

1979

# Hybrid poplar mycorrhizae and endogonaceous spores in Iowa

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HYBRID POPLAR MYCORRHIZAE AND ENDOGONACEOUS SPORES IN  
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Hybrid poplar mycorrhizae and endogonaceous spores in Iowa

by

Christopher Walker

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of  
The Requirements for the Degree of  
DOCTOR OF PHILOSOPHY

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## TABLE OF CONTENTS

	Page
GENERAL INTRODUCTION	1
Explanation of dissertation format	1
The poplar intensive culture program and problems addressed	2
An introduction to mycorrhizae	4
Mycorrhizae of poplars	26
Reports of investigations into the mycorrhizal status of poplars	28
The Endogonaceae	41
Population dynamics and species diversity of endogonaceous spores	84
PART 1. MYCORRHIZAE AND ASSOCIATED FUNGAL SPORES IN SEVEN HYBRID POPLARS POTTED IN FIVE IOWAN SOILS	98
INTRODUCTION	100
MATERIALS AND METHODS	101
RESULTS	106
DISCUSSION	115
Mycorrhizae	115
Fungal species	118
CONCLUSIONS	121
ACKNOWLEDGMENTS	123
LITERATURE CITED	124
PART 2. POPULATIONS OF ENDOGONACEOUS FUNGI IN TWO LOCATIONS IN CENTRAL IOWA	127
INTRODUCTION	129

	Page
MATERIALS AND METHODS	131
RESULTS AND DISCUSSION	138
Species diversity and distribution	140
Treatment effects	149
Population dynamics	154
Spore numbers	158
CONCLUSIONS	164
ACKNOWLEDGMENTS	166
LITERATURE CITED	167
PART 3. <u>COMPLEXIPES MONILIFORMIS</u> : A NEW GENUS AND SPECIES TENTATIVELY PLACED IN THE ENDOGENACEAE	170
INTRODUCTION	172
<u>COMPLEXIPES MONILIFORMIS</u> Walker gen. et sp. nov.	173
Distribution and habitat	176
Mycorrhizal associations	176
Etymology	176
DISCUSSION	178
ACKNOWLEDGMENTS	180
LITERATURE CITED	181
PART 4. <u>ACAULOSPORA SPINOSA</u> : A NEW SPECIES IN THE ENDOGENACEAE	182
INTRODUCTION	184
<u>ACAULOSPORA SPINOSA</u> Walker & Trappe sp. nov.	185
Distribution and habitat	190
Mycorrhizal associations	190

	Page
Etymology	190
Collections examined	191
DISCUSSION	192
KEY TO THE SPECIES OF <u>ACAULOSPORA</u>	193
ACKNOWLEDGMENTS	195
LITERATURE CITED	196
PART 5. SPECIES IN THE ENDOGONACEAE: <u>GLOMUS OCCULTUS</u> sp. nov. AND <u>GLOMUS GEOSPORUS</u> stat. nov.	197
DESCRIPTION OF THE NEW SPECIES	199
<u>Glomus occultus</u> Walker sp. nov.	199
Distribution and habitat	204
Mycorrhizal associations	204
Etymology	205
Collections examined	205
DISCUSSION OF <u>GLOMUS OCCULTUS</u>	206
THE NEW STATUS	208
<u>Glomus geosporus</u> (Nicolson & Gerdemann) Walker stat. nov.	209
DISCUSSION OF <u>GLOMUS GEOSPORUS</u>	213
ACKNOWLEDGMENTS	217
LITERATURE CITED	218
PART 6. <u>GLOMUS ALBIDUS</u> : A NEW SPECIES IN THE ENDOGENACEAE	219
INTRODUCTION	221
<u>GLOMUS ALBIDUS</u> Walker & Rhodes sp. nov.	222
Distribution and habitat	225



	Page
Mycorrhizal associations	225
Etymology	226
Collections examined	226
DISCUSSION	227
LITERATURE CITED	231
OVERALL SUMMARY AND DISCUSSION	232
General	232
Poplar mycorrhizae	232
Population diversity of endogonaceous spores	233
Effects of site preparation on endogonaceous spores	234
Distribution and dynamics of endogonaceous spore populations	234
Species of Endogonaceae in Iowa	236
LITERATURE CITED	240
ACKNOWLEDGMENTS	260

## GENERAL INTRODUCTION

Explanation of dissertation format

This dissertation is written following the alternative format approved by the Graduate Faculty of Iowa State University on 25 May 1979, whereby papers prepared for submission to scholarly journals may be sandwiched between a general introduction and a summary-discussion.

The papers included in this work fall into several parts. The first, on the mycorrhizae and associated endogonaceous spores of some hybrid poplars planted in different Iowan soils, was the result of an opportunistic survey of an experiment already developed for other reasons by the junior author (McNabb), who also assisted in the collection of roots for later laboratory examination. I assessed species and populations of spores, and analyzed the mycorrhizal status of the roots. The second part represents a population dynamics study of spores of the fungal family Endogonaceae in two field sites in central Iowa. In this case, I designed the surveys and did the field and laboratory work. Materials, travel, and technical support were provided by McNabb under the project on plantation establishment of intensive tree cultures (Iowa Agriculture and Home Economics Experiment Station). Advanced statistical advice was provided by the other junior author (Mize). The remaining parts deal with the taxonomy of the Endogonaceae. One new genus and four new species in this group are described, and one variety raised to

species status. This work became necessary when taxa not formally described in the literature were discovered during the surveys. Dr. James M. Trappe, of the Pacific Northwest Forest and Range Experiment Station, United States Forest Service, Corvallis, Oregon, is joint author of one of the species, since he discovered it in Mexico in 1972, but had not published its description when I found it in Iowa. He kindly agreed to the joint description despite his right of priority. Similarly, Dr. Landon H. Rhodes, Ohio State University, Columbus, Ohio, had found and pot cultured another of the species prior to its discovery in my research plots. For both joint papers, I wrote the description and, after obtaining comments from the co-author and other reviewers, completed the typescripts for submission to Mycotaxon.

#### The poplar intensive culture program and problems addressed

The Department of Forestry at Iowa State University (ISU) is cooperating with the North Central Forest Experiment Station of the United States Forest Service in development of intensive culture methods for economically producing large quantities of wood fiber from Populus species (U.S. Forest Service, 1976). Part of this program is aimed at understanding interactions in the root zone, and using this understanding to improve cultural methods and hence increase yield (Haywood, 1978; Lee & Promnitz, 1978).

One factor in the root zone that can be of great importance to the establishment and vigor of trees is the symbiosis between fungi

and roots -- mycorrhizae. Wilde (1968) suggested that, in nature, there may be no such thing as a non-mycorrhizal tree. Many studies have shown that, in general, mycorrhizal fungi are greatly beneficial to their hosts (see reviews by Gerdemann, 1968; Hacskeylo, 1972; Harley, 1959; Kormanik et al., 1977a; Mosse, 1973; and Trappe, 1977a). This has been shown to be especially true for the early growth stages of some hardwoods, and for yellow poplar (Liriodendron tulipifera L.), sweetgum (Liquidambar styraciflua L.), and red maple (Acer rubrum L.), symbiosis may be obligatory, at least in normal nursery soils (Bryan & Kormanik, 1977; Bryan & Ruehle, 1976; Clark, 1964; Kormanik et al., 1976, 1977b, 1977c, 1978; Marx, 1977). Poplars do not have such an obligate requirement, as is shown by the ready rooting and excellent growth in a non-mycorrhizal condition in the greenhouse and growth chambers. Poplars in the field, however, are usually mycorrhizal (Dominik, 1958; Trappe, 1962) and the question is therefore what function do mycorrhizae have in the ecology of these plants.

Little is known about poplar mycorrhizae and there are only two reports relating to their development in the field and effects on growth as outplants become established (Harris & Jurgensen, 1977; Mejstrik, 1971). Nothing was known of the species and biology of fungi mycotrophic with poplars used in the intensive culture program. Because basic knowledge of the biology of a crop is a prerequisite to optimum management, work commenced in 1975 to study poplar mycorrhizae. The work addressed in this dissertation relates mainly to population biology and species diversity of fungi in the family

Endogonaceae associated with poplars used for intensive culture.

The questions raised were

- 1) Are poplars used for intensive culture mycorrhizal?
- 2) What types of mycorrhizae are found on these poplars, and is it possible to identify the fungal symbionts?
- 3) How do populations of possible endomycorrhizal symbionts change in the field during the initial establishment of intensive culture plantations of poplars?

### An introduction to mycorrhizae

General The term "mycorrhiza" -- literally "fungus root" -- was coined by A. B. Frank in 1885 (Frank, 1885 as reviewed in Kelley (1937)) to give a concise and elegantly accurate descriptive name to the symbiosis between plant roots and various soil inhabiting fungi. Although the term originally applied only to those mycorrhizae that are now known as ectomycorrhizae, Frank later extended his definition to include the various endotrophic forms (Frank, 1887 as reviewed in Kelley (1937)). Mycorrhiza is now applied to all mycotrophic symbioses between plant roots and fungi (Harley, 1959).

Mycotrophy is a symbiotic association between a plant and a fungus which benefits the plant. The relationship also may benefit the fungus, but may eventually be detrimental to it. Sometimes, mycorrhizal fungi may be harmful to plants, but such instances are not mycotrophies (Kelley, 1950).

The word "symbiosis" is defined simply by its originator

(de Bary, 1878 as reviewed in Kelley (1937)) as a living together of two dissimilar organisms. Of itself, the word has no connotations regarding benefit to members of the symbiosis. A qualifying adjective is required to make clear where benefit, if any, lies (Table 1).

Table 1. The hypothetical different types of symbiotic association between fungi and plants<sup>a</sup>

Type	Interaction <sup>b</sup>		Notes
	Plant	Fungus	
Neutralistic	0	0	
Mutualistic	+	+	Obligate
Protocooperative	+	+	Optional
Commensal	+	0	or 0 +
Competitive	-	-	Antagonistic
Amensal	-	0	or 0 -
Pathogenic	-	+	
?	+	-	e.g., orchidaceous mycorrhizae

<sup>a</sup>Modified from the writings of Odum, 1959.

<sup>b</sup>0 = no effect. + = beneficial. - = detrimental.

Another type of relationship, not mutually exclusive with those above is parasitism. A parasite is an organism (the parasite) that gains food and structural support (and transportation in the case of

some animals) for a considerable period of time from another (usually larger) organism (the host) (Open University, 1974). A parasite, by this definition, need not be a pathogen. The interaction between plant and fungus may be mutualistic, protocoperative, commensal, or pathogenic. By this definition, mycorrhizal fungi are parasitic, even though they may be greatly beneficial to the host plant.

Although Frank, with amazing insight, considered mycorrhizae to be beneficial to the plant, their study was hampered for many years by the opinions of Robert Hartig, who considered the fungi involved to be weakly pathogenic and to "... lessen activity of the new roots, or even lead to their death ..." (Hartig, 1886; 1888a; 1888b all as reviewed in Kelley (1937)). Hartig's influence was so great that, despite considerable evidence to the contrary, even as late as 1939, many people shared his view (e.g., Burgess, 1936; Curtis, 1939). Frank's hypothesis eventually prevailed (e.g., Beattie, 1976; Bergstrom, 1976; Kormanik et al., 1977a), and although there is little doubt that under some circumstances a mycorrhizal fungus may become pathogenic or depress growth (Crush, 1976; Gerdemann, 1968; Hall, 1977; Hall et al., 1977, Harley, 1959, Johnson, 1976), or may be neutralistic (Hall, 1974), in the majority of instances the mycorrhizal fungus is either mutualistic or protocoperative.

Mycorrhizae, then, are inseparable combinations of fungi and roots. They are, by definition, beneficial to the plant, and are therefore mycotrophies. They may benefit the fungus, or be seemingly neutral or detrimental to it. On some occasions, the fungus may harm

the plant, but in such cases, the symbiosis is not a mycotrophy. Mycorrhizal fungi can be regarded as parasites, and their symbionts considered their hosts.

Types of mycorrhizae      Details of the different kinds of mycorrhizae can be found in the works of Harley (1959), Hatch (1937), Kelley (1950), Marks & Kozlowski (1973), and Sanders et al. (1975). The remainder of this General Introduction is an attempt to present only the salient points of mycorrhizal structure and function.

There are five different classes of mycorrhizae presently recognized (Lewis, 1975; Peyronel et al., 1969; Wilcox, 1971) (Table 2). Ericaceous and orchidaceous mycorrhizae are restricted, as their names imply, to the Ericales and Orchidales respectively. Ericaceous mycorrhizae are apparently formed by species of Pezizella (Read & Stribley, 1975), and by various basidiomycetes and the sterile Cenococcum geophilum Fr. (Zak, 1976a; 1976b). Orchidaceous mycotrophies are formed by members of the form genus Rhizoctonia, some of which may belong to the basidiomycete genera Tulasnella and Ceratobasidium (Hadley, 1975; Hadley & Ong, 1978). Further comments on these two groups of mycorrhizae are not appropriate in this dissertation on mycorrhizae of poplars.

The description of the three remaining groups, however, is appropriate. All three were found on poplars in the course of studies reported in this dissertation.

Ectomycorrhizae      These are the best known mycorrhizal types,



Table 2. The groupings of mycorrhizae

Natural class	Alternate names	Group <sup>a</sup>
Ectomycorrhiza <sup>b</sup>	(Ectotrophic (Sheathing (Hartigian	} Ectomycorrhiza
Ericoid ericaceous <sup>b</sup>	(Endotrophic (Ericaceous	
Orchidaceous <sup>b</sup>	Endotrophic	} Endomycorrhiza
Arbuscular endomycorrhiza <sup>b</sup>	(Endotrophic (Phycomycetous (Vesicular-arbuscular (Arbuscular (Zygomycetous (Endogonaceous	
Arbutoid ericaceous <sup>b</sup>		
Ectendomycorrhiza <sup>c</sup>		

<sup>a</sup>Of Peyronel et al. (1969).

<sup>b</sup>Of Lewis (1975).

<sup>c</sup>Of Mikola (1965), and Wilcox (1971).

although only about 3% of plants form them. In the gymnosperms they are common in all species of Pinaceae and some Cupressaceae. In angiosperms they tend to be restricted to large woody perennials. Most of the Fagales; Populus and Salix in the Salicales; Ulmus in the Urticales; Crataegus, Malus, Pyrus, and Sorbus in the Rosales; several woody Leguminosae; and Acer in the Aceraceae are ectomycorrhizal. In addition, Tilia in the Malvales and Eucalyptus in the Myrtiflorae have ectomycorrhizal members (Meyer, 1973).

The majority of ectomycorrhizal fungi are Agaricales or hypogeous Gasteromycetes (Smith, 1971), although many hypogeous ascomycetes in the Elaphomycetales (Trappe, 1979) and Pezizales (ord. emend.; Trappe, 1979) are also ectomycorrhizal. Cenococcum geophilum (= C. graniforme (Sow.) Ferd. & Winge), a form species in the Mycelia Sterilia that almost certainly is related closely to Elaphomyces anthracinus Vitt. (Trappe, 1971), is a common ectomycorrhizal associate. At least one Zygomycete (Endogone lactiflua Berk.) is ectomycorrhizal with conifers (Fassi et al., 1969).

The ectomycorrhiza is morphologically different from an uncolonized root, and is, to some extent, recognizable macroscopically without any special staining or clearing techniques. It is, in its simplest form, a swollen root tip (Figure 1) over which the fungus forms a covering (the sheath or mantle), at the same time suppressing root hair formation. A network of hyphae, known as the "Hartig net", replaces the middle lamella of the epidermal and/or cortical cells of the root tip. Hartig net development may vary from shallow penetration

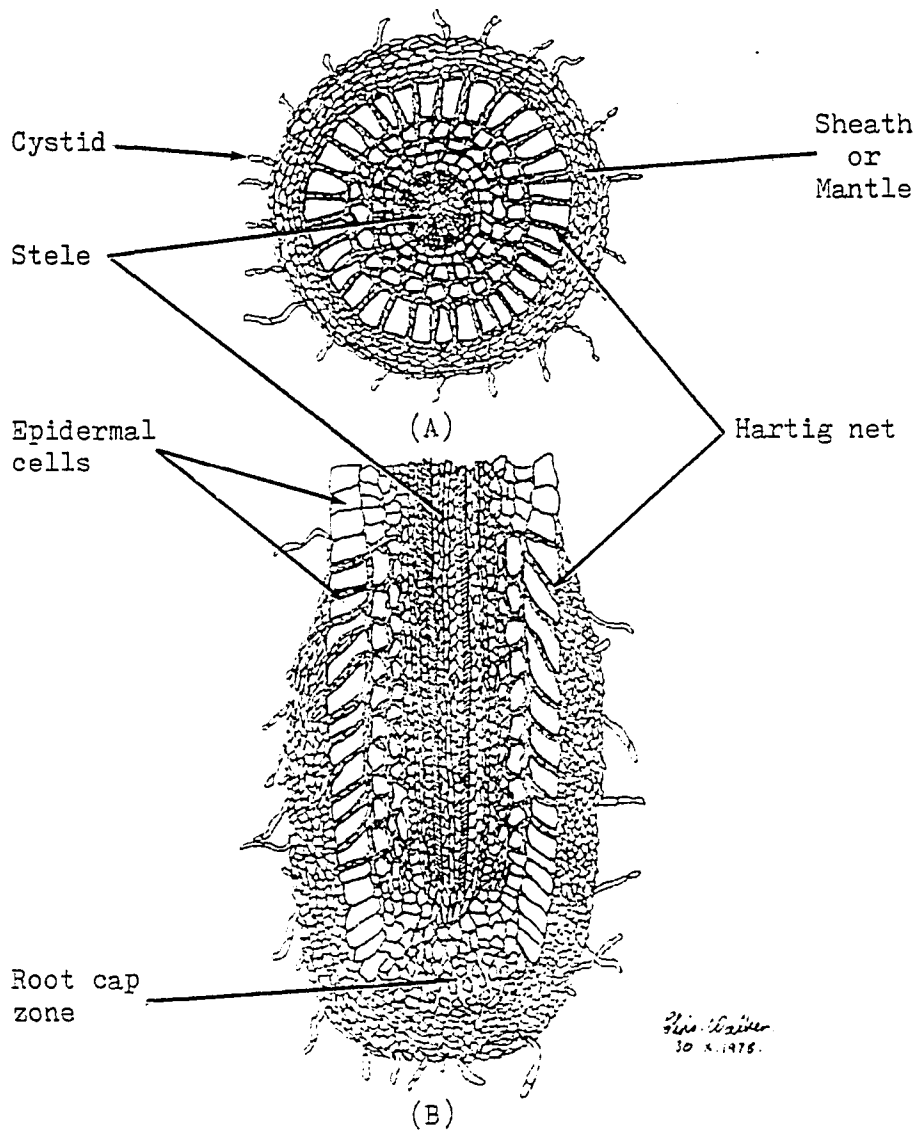


Figure 1. Cross section (A) and median longitudinal section (B) of a simple mycorrhiza (drawn from a white oak mycotrophy with an unknown symbiont)

as in Alnus glutinosa (L.) Gaertn. (Green, 1979), through penetration just into the outer cortex (often resulting in greatly elongated epidermal cells) as in Fagus sylvatica L. (Harley, 1959), to an extensive colonization that may almost reach the endodermis. A mycorrhiza with this last feature was pictured for Pinus mughus Scop. by Boullard (1968).

Ectomycorrhizae are extremely varied morphologically, and the shape of the root tip, even in the simplest unbranched forms, is changed. In pines the short roots often become bifurcate or proliferate into clusters. In other conifers, especially Douglas fir (Psudotsuga menzeisii (Mirb.) Franco), pinate mycorrhizae form, or the short roots proliferate into tight bundles enclosed in a thick fungal layer, to form tuberous structures.

Ectomycorrhizae have greatly differing degrees of hyphal proliferation in soil. The surface of the sheath may be smooth, there may be short cystidia or setae, or there may be extensive mycelial mats, strands, or rhizomorphs which ramify far into the soil. The significance of this variation is unknown. Melin (1927 as cited in Zak (1971)) and Dominik (1956; 1959) presented schemes for classifying the ectomycorrhizae on morphological features. Zak (1971) considered these schemes unsatisfactory and presented a hierarchal method of characterization based on (1) both symbionts' binomials, (2) host binomial and fungal isolate designation, or (3) host binomial and mantle description. Zak also used such factors as color of mantle, morphology of mycorrhiza, fluorescence of fungus, and reactions to

chemical reagents. His hope that the system would lead to a key for the identification of ectomycorrhizal symbioses has not yet been realized.

As mentioned earlier, ectomycorrhizae, to some extent, can be recognized macroscopically, but caution should be exercised when doing so. Some fungi form sheaths around root tips without penetrating between cells to form a Hartig net, and without benefitting the plant. Other species of fungi are weakly pathogenic, forming a sheath and intracellular haustoria. Yet other species of fungi are parasitic on true mycorrhizae, forming a secondary sheath around the mantle and perhaps leading to the demise of the organ (Harley, 1959). Non-mycorrhizal roots may be induced to take on a mycorrhizal appearance by treatment with certain regimes of light and plant growth hormones (Slankis, 1971; 1973). Such morphological changes in experimental material may result in misleading observations. Therefore to be sure of the ectomycorrhizal nature of any organs observed, stained sections or root clearings should be made to confirm the presence of a Hartig net.

Endomycorrhizae Only endotrophic mycotrophies of the type formed by asexual members of the family Endogonaceae (Mucorales: Zygomycetes) will be considered in this subsection, and the term "endomycorrhiza" will be used solely in that sense in the remainder of this dissertation, unless otherwise stated.

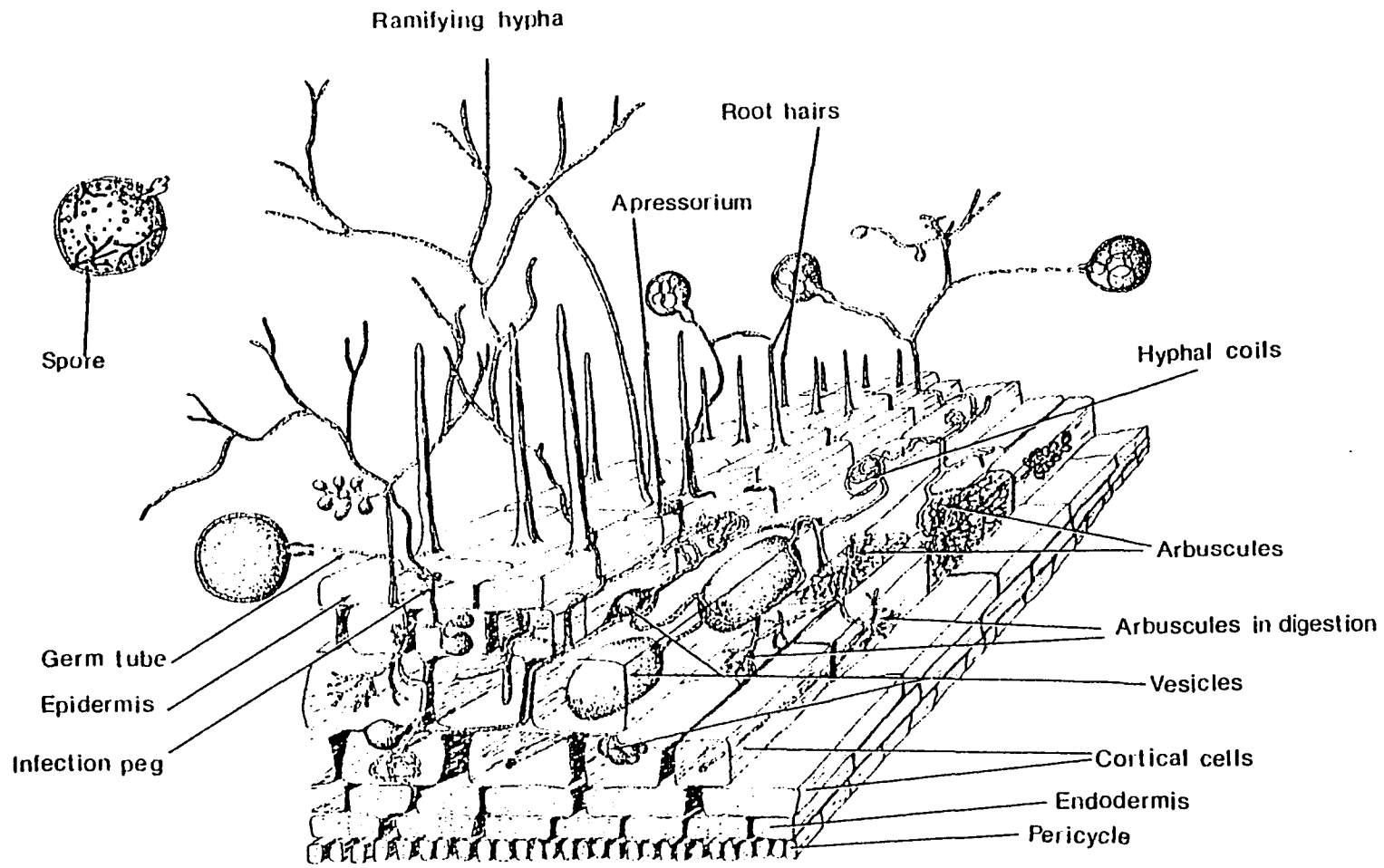
The vast majority of rooted vascular plants form endomycorrhizae with certain members of the Endogonaceae. As Gerdemann (1968)

commented, it is easier to list the plant families without these symbioses than those that have them. Gerdemann's list showed the Cyperaceae, Commelinaceae, Juncaceae, Urticaceae, Polygonaceae, Chenopodiaceae, Amaranthaceae, Nyctaginaceae, Phytolaccaceae, Aizoaceae, Portulacaceae, Caryophyllaceae, Fumariaceae, and Cruciferae, along with most aquatic plants, to be non-mycotrophic. Although some members of these groups are endomycorrhizal (Bagyaraj et al., 1979a; Fontana, 1963a; Kouckeki & Read, 1976; Mejsstrik, 1965; Sondergaard & Laegaard, 1977), they are in general free of mycotrophic partners. Heavily ectomycorrhizal plants are not normally endomycorrhizal (e.g., the Pinaceae and ectotrophic Fagales) (Gerdemann, 1968), but some trees (e.g., Alnus, Populus, and Sambucus can have both types, even on the same root system (Dominik et al., 1954; Fontana 1961a, 1961b, 1962; Green, 1979; Hall et al., 1979). Endomycorrhizae on Pinus halepensis Mill. may be phycomycetous, and at least one species of orchid may have such endomycorrhizae (Hall, 1976).

In contrast to the ectomycorrhizae, endomycorrhizae do not form a thick hyphal mantle or Hartig net, and at least some of the mycobiont's hyphae penetrate the plant cells to form intracellular structures. There is no readily noticeable change in the morphology of the roots (Gerdemann, 1968), although sometimes they turn from white to bright yellow upon establishment of the symbiosis (Furlan & Fortin, 1973).

Figure 2 is a diagrammatic representation of the structure and development of a phycomycetous endomycorrhiza. The spore germinates

Figure 2. Diagrammatic representation of a phycomycetous (vesicular-arbuscular) endomycorrhiza





by a long germ tube which grows through the soil. Initially, the germ tube grows at random, but if it comes close to a root suitable for colonization, its growth becomes directed towards the root (Mosse & Hepper, 1975; Powell, 1976a). The mechanism causing and operating this behaviour is unknown, but hyphae grow over and past some roots. Presumably the triggering mechanism, possibly root exudation, is not present in such instances. On contacting a suitable root, an appressorium is formed and an infection peg penetrates an epidermal cell, sometimes by way of a root hair. The hypha branches and grows into, through, and between the cortical cells. Most growth is in the middle layers of cortex, though structures may be formed throughout the cortex (Nicolson, 1967), but not in the stele. The hyphae often form extensive coils inside the cells. Haustorium-like structures are formed by dichotomous branching of side branches. These "arbuscules" (Gallaud, 1905 cited in Butler (1939)) function in nutrient exchange between plant and fungus (Cox et al., 1975; Cox & Tinker, 1976; Schoknecht & Hattingh, 1976). The arbuscules eventually collapse and are digested by the host, forming small sac-like structures formerly called "sporangioles" (Janse, 1897 as reviewed in Kelley (1937)), but now known to be simply a phase in the demise of arbuscules (Kinden & Brown, 1976). Finally, large vesicles may form within and between cells. Vesicles are apparently storage organs, although they also may convert into resting spores. The formation of arbuscules and vesicles gives rise to the vernacular "vesicular-arbuscular" mycorrhiza, although vesicles

are absent in some species (Daft & Nicolson, 1974; Gerdemann & Trappe, 1974).

Besides the internal hyphae, there is considerable growth of the fungus in the soil. This effectively increases the absorbing surface available to the plant for nutrient uptake (Rhodes & Gerdemann, 1975). Large sporocarpic or ectocarpic resting spores may form on the external hyphae, and in some endogonaceous fungi, spores form within the root cortex (Ames & Linderman, 1976; Gerdemann, 1955; Gerdemann & Nicolson, 1963). Some mycobionts may not form spores, especially when associated with perennial hosts (Baylis, 1969; Crush, 1975; Hall, 1977).

Reviews of phycomycetous endomycorrhizae by Gerdemann (1968), Mosse (1973), and Nicolson (1967), while somewhat out of date, still give an excellent base for the study of these organs. Although Harley (1975) has chided that "... mycorrhizasts spend too much time reviewing their subject ...," the output of papers on phycomycetous endomycorrhizae is currently so great that another review would be welcome.

Ectendomycorrhizae Mikola (1965) described a form of mycotrophy of forest trees that differs from ectomycorrhizae in having extensive intracellular hyphal structures; a mantle that may be extremely thin, or sometimes lacking, and usually is not observable without a microscope; and a definite Hartig net. He explained that considerable confusion arose from previous descriptions of both

mycotrophic and weakly pathogenic organisms as ectendomycorrhizal.

Harley (1959) suggested that the differences existing between ectomycorrhizae and ectendomycorrhizae have been overemphasized, and this was reiterated by Marks & Foster (1973). Wilcox et al. (1974) acknowledged Harley's statement, saying " ... it is accepted ... ectomycorrhizae have intracellular infections to varying degrees, but these are not a predominating feature [my emphasis]." It seems clear from the work of Mikola (1965) and Wilcox and his associates (Wilcox, 1971; Wilcox & Ganmore-Neumann, 1974; Wilcox et al., 1974) that consideration of these structures as a separate group of mycorrhizae is sound on morphological, anatomical, and ecological grounds, at least pending much more investigation.

The ectendomycorrhizae of the Ericaceae should be termed "arbutoid ericaceous mycorrhizae" (Table 2) and the term "ectendomycorrhiza" should be reserved for structures with the following characteristics:

- 1) The morphology of the roots may be changed as with some ectomycorrhizae, but also may be little or no different from a non-mycorrhizal root.
- 2) There is a Hartig net, from which intracellular hyphae penetrate the cortical cells without causing injury.
- 3) There may be a mantle, though sometimes it will be absent. The mantle is usually very thin (4-10  $\mu\text{m}$ ) and smooth, and not visible to the unaided eye.
- 4) In contrast to the ectomycorrhiza, both long and short roots are colonized.

Wilcox and his associated workers have cultured a number of fungi known to cause ectendomycorrhizae with pines, but the identity of most remains a mystery. However, one has been placed provisionally in the Endogonaceae (Walker, 1979), and another is referred to as a possible Chloridium species. Chloridium is an imperfect fungus in the Phialosporae (Barron, 1972). Froidevaux & Amiet (1975a, 1975b) reported ectendomycorrhizae on pines, formed by species of Suillus, Boletus, and Rhizopogon, but whether these were true ectendomycorrhizae as described here, or ectomycorrhizae with some intracellular hyphae is not clear.

Ectendomycorrhizae seem to predominate on young trees (1-3 years old) in disturbed sites such as forest nurseries or in pots and greenhouses. They are replaced by ectomycorrhizae shortly after outplanting of the transplants, except on severely disturbed sites. Thus ectendomycorrhizae are ecologically distinct, their mycobionts appearing unable to compete in established ecosystems, but perhaps functioning in pioneering plant communities. Much more work is needed before the controversy over these structures can be resolved. As Marx & Bryan (1975a) wrote, "Very little is known about their significance to tree growth, since only limited research has been attempted on them."

Mycorrhizal functions      All types of mycorrhizae benefit the host plant in some way, usually improving growth, particularly in low nutrient soils (e.g., Crush, 1974; Kleinschmidt & Gerdemann, 1972;

Laiho, 1966; Marx & Bryan, 1975b; Mosse, 1977; Park, 1970; Possingham & Groot Obbink, 1971; Powell, 1976b; Saif & Khan, 1977; Voigt, 1971; Vozzo & Hacskeylo, 1971), although sometimes fungi that are normally mycorrhizal can become a drain on the plant (Crush, 1976; Fox & Spasoff, 1972; Levisohn, 1954) where available phosphorus level is high. In general, they aid the plant in nutrient uptake, especially of relatively insoluble elements. Of these elements, phosphorus is the most important because of its high demand in plant metabolism, and its immobility in most soils. Many studies have been done on the role of mycorrhizae in phosphorus uptake (e.g., Baylis, 1967; Daft & Nicolson, 1969; Hall et al., 1977; Hayman et al., 1975; Johnson, 1976; Mejstrik, 1975; Malajczuk et al., 1975; Timmer & Leyden, 1978).

Uptake of other elements also is enhanced. Endomycorrhizae can prevent zinc deficiency symptoms from appearing in peach on soils with inadequate supplies of that element (Gilmore, 1971). Zinc uptake was demonstrated in Pinus ectomycorrhizae and Araucaria endomycorrhizae (Bowen et al., 1974). Higher sulfur uptake was shown in onions and clover mycorrhizal with Glomus fasciculatus (Thaxter sensu Gerdemann) Gerdemann & Trappe (Rhodes & Gerdemann, 1978a; 1978b) Soybeans mycorrhizal with Gl. mosseae (Nicolson & Gerdemann) Gerdemann & Trappe absorbed more strontium than did non-mycorrhizal plants, leading to the suggestion that cations such as calcium and magnesium would behave similarly (Jackson et al., 1973). Ross (1971) and Routien & Dawson (1943) showed that, for endomycorrhizae and ectomycorrhizae respectively, a general enhancement of nutrient uptake

was evident; calcium, magnesium, iron, potassium, phosphorus, and nitrogen being mentioned in these studies.

There is some controversy over the way that mycorrhizae enhance nutrient uptake. In general it is believed that they do not solubilize normally unavailable phosphorus, but instead operate by extending the volume of soil from which the element can be extracted (Barrow et al., 1977; Hayman & Mosse, 1972). This is the result of increased absorptive surface, brought about by the exploration of soil by fungal hyphae (Hattingh et al., 1973; Owusu-Bennoah & Wild, 1979; Skinner & Bowen, 1974). However, Voigt (1971) believed that, beyond this increase in physical surface, the fungi produce acids able to break down silicates in the soil, and hence make otherwise immobile elements available. Stark (1971) has even suggested that mycorrhizal fungi can directly break down litter in extremely nutrient-poor soils such as are found in tropical forests, but proof is lacking. Ryegrass (Lolium perenne L.), mycorrhizal with Glomus tenuis (Greenall) Hall and Gigaspora spp., absorbed phosphorus from an insoluble source (Nauru rock phosphate), whereas autotrophic plants did not (Powell, 1976b; Powell & Daniel, 1978). Surface phosphatase activity detected on mycorrhizae of beech would allow the use of complex organic and inorganic sources of phosphorus (Bartlett & Lewis, 1973). The controversy is not yet settled, and much more research is required in this area.

Besides enhancing nutrient uptake, mycorrhizal fungi play other roles in the soil. Endomycorrhizal fungi are important in binding soil particles in sand dunes, thus helping the pioneer grasses to

fix the shifting sands (Sutton & Sheppard, 1976; Clough & Sutton, 1978). Water relationships are improved for both ectomycorrhizal and endomycorrhizal plants (Safir et al., 1971, Sands & Theodorou, 1978; Uhlig, 1973). Linderman & Call (1977) described improvement of rooting for ericaceous cuttings colonized by a suitable mycobiont. Leaching of inorganic nitrogen from a soil column was retarded by Gl. mosseae in association with Liquidambar styraciflua (Haines & Best, 1976). Pinus nigra Arn. ssp. nigricans Host could only grow on high calcium sites if the trees were ectomycorrhizal (Clement et al., 1977).

Zak (1964), in a largely speculative work, reviewed the literature pertaining to the role of mycorrhizae in resistance to root disease, and concluded that the ectomycorrhizal mantle would be expected to provide considerable protection against pathogens. A review by Marx (1971) reported evidence, supporting Zak's hypothesis, on production of antibacterial and antifungal compounds by ectomycorrhizae, and on the efficacy of the physical barrier formed by the mantle and Hartig net. He reviewed the subject once again (Marx, 1973) and reached the same conclusions. Since then, further evidence has accumulated. Endomycorrhizae have been shown to give protection to plants against Fusarium and Thielaviopsis, though the mechanism of this is unclear (Dehne & Schonbeck, 1975; Schonbeck, 1978). Damping off in rape (Brassica napus L.), caused by Rhizoctonia solani Kuhn was greatly reduced by endomycorrhizal colonization (Iqbal et al., 1977). This is especially interesting

in view of the fact that crucifers are generally non-mycorrhizal. Gomphidius glutinosa (Schaeff. ex Fr.) Fr., a fungus probably mycorrhizal with spruce, was highly antagonistic in vitro to the root rot fungus Heterobasidion annosum (Fr.) Bref. (= Fomes annosus (Fr.) Karst.), and Suillus placidus (Bon.) Sing. was moderately antagonistic to the same pathogen (Froidevaux & Amiet, 1974).

Resistance to nematodes also has been attributed to mycorrhizal formation. Establishment of mycorrhizae in cotton by Gigaspora margarita Becker & Hall nullified stunting caused by the root-knot nematode Meloidogyne incognita Chitwood (Roncadori & Hussey, 1977), and the same nematode on tomatoes was affected adversely when colonization by Glomus mosseae took place (Sikora, 1978). Sikora suggested that the latter was caused by changes in host physiology induced by mycorrhizal establishment.

A few studies have shown somewhat different results. Heterodera solanacearum Miller & Gray and Gigaspora gigantea (Nicolson & Gerdemann) Gerdemann & Trappe mutually suppressed each other's reproduction, and both could reduce plant growth (Fox & Spasoff, 1972). The lance nematode (Hoplolaimus galeatus (Cobb)) was not prevented from attacking pine roots by the mycorrhizal mantle (Ruehle & Marx, 1971), and a similar report for a Meloidogyne sp. on Pinus ponderosa Laws. (Riffle, 1973) indicated that rupture of the sheath by the nematodes allowed entry of pathogens. Increased virus production in mycorrhizal plants of tomato, petunia, and strawberry was paralleled in non-mycorrhizal plants supplied with large amounts



of phosphate. The mycorrhizal plants nevertheless grew better than controls.

Mycorrhiza is a 2-membered symbiosis only when viewed from our rather limited experimental point of view. Actually, a myriad of other rhizosphere and rhizoplane organisms occur, and the complete web of interactions is but minimally understood. Bacteria have been detected in the root caps of endomycorrhizae (Bonfante & Scannerini, 1974/75), and there is evidence of a synergism between endomycorrhizae and phosphate-solubilizing bacteria (Pseudomonas sp. and Agrobacterium sp.), especially on low phosphorus alkaline soils (Azcon et al., 1976; Barea et al., 1975). A synergistic or additive interaction between Glomus fasciculatus and Azotobacter chroococcum may exist in tomatoes (Bagyaraj & Menge, 1978).

The most commonly investigated tripartite interaction has been that of legumes, where Rhizobium and endomycorrhizal fungi interact together and with the host plant. Mycorrhizae appear to increase nodulation and nitrogen fixation, and dually colonized plants produce more fruits, dry matter, and nodules (Bagyaraj et al., 1979b; Daft & El-Giahmi, 1974, 1976; Godse et al., 1978; Mosse et al., 1976; Smith & Daft, 1977). Green (1979) examined Alnus glutinosa in symbiosis with a basidiomycete ectomycorrhizal fungus (species unknown) and a non-legume endophyte (Frankia sp.). The tripartite system produced superior height growth when compared with seedlings without symbionts, or with only one. The addition of endomycorrhizae to this tripartite system would make an interesting study. There is

considerable potential for work in this fascinating area of multi-membered symbioses.

The benefits of the mycorrhizal symbiosis are not all unidirectional. Most mycorrhizal fungi are fastidious with regard to nutrients; indeed, only a few are amenable to truly axenic culture<sup>1</sup>. They gain their carbon supply in a readily available form from the plant, along with vitamins and other essential organic compounds, which they are unable to synthesize unaided (Kelley, 1950). These fungi also find an ecological niche for survival. Plants within an ecosystem may be linked by mycorrhizal fungi (Reid, 1971), and this may be important in overall nutrient cycling. The biology and physiology of carbon transfer in mycorrhizae has been reviewed by Bevege et al. (1975), Lewis (1975), Purves & Hadley (1975), Stribley & Read (1975), and Woolhouse (1975) in a series of papers presented at the Symposium on Endomycorrhizae held at Leeds University, England, in July 1974 (Sanders et al., 1975).

To conclude this introduction to mycorrhizae, the following quotation from Trappe & Fogel (1977) clearly expresses my own beliefs:

"As key links in belowground nutrient and energy cycling, mycorrhizae and their mycobionts can be ignored only at

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<sup>1</sup>In many papers on mycorrhizae, the growth of mycorrhizal fungus and plant together in vitro is referred to as an "axenic culture." Axenic, strictly speaking, means "without or deprived of any commensals or symbionts" and the term "gnotobiotic" more properly should be used (see Kenneth, 1963).

substantial peril of reaching unreal conclusions about ecosystem processes."

This is an admonition that all biologists and ecologists working with plants would do well to consider.

### Mycorrhizae of poplars

General Although observations on mycorrhizae of poplars were made as long ago as the turn of the century (Dangeard, 1896; Stahl, 1900 as cited in Kelley (1950)), the literature on poplar mycotrophy is still not comprehensive. Dominik (1958) referred to a review of Populus mycorrhizae by Kelley (1937 as cited in Dominik (1958)), but I have searched in vain for a copy of this mimeographed item. Dominik's works on the subject (1956, 1958) are the most detailed studies available, and little has been published since. He recently co-authored a paper (in Polish) on fungal associates of poplars (Dominik & Ihnatowicz, 1979), the only recent work on poplars cited being that of Vozzo & Hacskeylo (1974).

Fungal symbionts Trappe (1962) listed 44 papers in which known fungal species are mentioned as probably ectomycorrhizal symbionts with poplars. Thirty-one fungi were listed from among 10 named poplar taxa and unnamed Populus species. Only three had been proved mycorrhizal by pure culture methods: C. geophilum, Leccinum aurantiacum (Bull.) S. F. Gray, and L. scabrum (Bull. ex Fr.) S. F. Gray, all with P. tremula L. (Lihnell, 1942; Melin, 1923; both as

cited in Trappe (1962)). Since these studies, only five papers have referred ectomycorrhizae of poplars to known fungal species. Among these were five previously unknown mycotrophies, but no additional poplar species were involved, and only one relationship, Tuber albidum Pico with P. x euramericana (Dode) Guinier was synthesized in vitro.

Trappe did not survey the literature on endomycorrhizae, but had he done so, he would have found only two references to a named fungus endomycorrhizal with poplars (Dangeard, 1896; 1900). These papers are historically interesting in that they reported investigations from which the first formal description of a fungus causing vesicular-arbuscular mycorrhizae was made. Later knowledge proved Dangeard's Rhizophagus populinus to be a description of a typical vesicular-arbuscular mycorrhiza, and the fungus involved cannot be placed to species. Possibly it was a Glomus sp., but it could have been from the genus Sclerocystis, or perhaps even from Acaulospora.

Vozzo (1969) considered that he was reporting endomycorrhizae on P. deltoides Bartr. for the first time, but he was actually preceded in this by more than four decades by Lohman (1927), who, in a study of mycorrhizal plants from parts of Iowa, reported the species to possess both ectomycorrhizae and phycomycetous endomycorrhizae. Neither report named the fungal symbionts, but a Glomus species is illustrated in Vozzo's paper. Later, Vozzo & Hacskeylo (1974) again associated Glomus species, and possibly a

Sclerocystis species, with endomycorrhizal cottonwood, though their illustrations are inadequate for positive identification. Shuja et al. (1971) reported "yellow vacuolate spores" (probably Gl. mosseae) associated with endomycorrhizal P. x euramericana in Pakistan.

Dominik (1958) showed that P. tremula, P. alba L., P. nigra L., P. nigra x P. alba, P. nigra var. italica Du Roi, P. x euramericana, P. x berolinensis Dippel, and P. x marilandica Bosc. were all endomycorrhizal, although his identification of the symbiont was R. populinus. His descriptions, however, make it clear that the genus Glomus was involved. Dominik & Ihnatowicz (1979) seem to relate Glomus macrocarpus Tul. & Tul to unnamed mycorrhizal poplars. I have listed all assumed or proven poplar mycobionts and their literature references in Tables 3-5.

#### Reports of investigations into the mycorrhizal status of poplars

From the works and reviews of Dominik (1958), Levisohn (1957), McDougall & Jacobs (1927), Mejstrik (1971), Melin (1923 as reviewed in Kelley (1937)), Trappe (1962), and Vozzo & Hacskeylo (1974), I compiled a list of the poplars that have been studied for mycorrhizal status (Table 6). Other works, such as Bartoli & Rambelli (1973), Borisova (1956 as reviewed in Review of Applied Mycology (1957) 36: 547), Shterenberg (1949 as reviewed in Review of Applied Mycology (1951) 30:282-283), and Thomas (1943) describe ectomycorrhizae from poplars. Fontana (1961a, 1961b) and Lohman (1927) also reported poplar endomycorrhizae, but neither adds new poplar species to the

Table 3. Known mycorrhizal fungal associates with unnamed Populus spp.

Fungal species	Literature reference (See Table 5)
<u>Amanita vaginata</u> (Bull. ex Fr.) Vitt. <sup>a</sup>	10, 29
<u>Cenococcum geophilum</u> Fr. <sup>a</sup>	25
<u>Cortinarius collinatus</u> (Pers. ex Fr.) Fr. <sup>a</sup>	15, 16
<u>Hebeloma longicaudum</u> (Pers. ex Fr.) Kumm.	10
<u>Inocybe decipientoides</u> Peck	10
<u>Laccaria laccata</u> (Scop. ex Fr.) Berk. & Br.	10
<u>Lactarius controversus</u> (Pers. ex Fr.) Fr. <sup>a</sup>	6, 10, 16, 19, 49
<u>Leccinum aurantiacum</u> (Bull.) S. F. Gray <sup>a</sup>	6, 15, 16, 40, 50, 51
<u>L. duriusculum</u> (Schulzer in Fr.) Sing. <sup>a</sup>	6
<u>L. nigrescens</u> (Rich. & Roze) Sing.	41
<u>L. scabrum</u> (Bull. ex Fr.) S. F. Gray <sup>a</sup>	15, 16
<u>Melanoleuca melaleuca</u> (Pers. ex Fr.) Murr.	10
<u>Paxillus involutus</u> (Batsch ex Fr.) Fr. <sup>a</sup>	10
<u>Russula pulchella</u> Borszcz.	14, 35
<u>Tricholoma irinum</u> (Fr.) Kumm. <sup>a</sup>	6
<u>T. pessundatum</u> (Fr.) Quel.	1
<u>T. populinum</u> J. Lange	15, 52
<u>Volvariella speciosa</u> (Fr.) Sing.	10
<u>Xerocomus chrysenteron</u> (Bull. ex St. Am.) Quel.	10
<u>Glomus macrocarpus</u> Tul. & Tul. (?)	9

<sup>a</sup>Known also from named poplar species (see Table 4).

Table 4. Known mycorrhizal fungal associates with named Populus spp. Underlined references refer to associations synthesized in vitro from pure cultures of the fungus

Poplar group (genus section (after Peace, 1952))		Literature reference (see Table 5)
Poplar species	Fungal species	
White (Leuce)		
<u>P. alba</u> L.	<u>Cenococcum geophilum</u> Fr.	6,10
	<u>Glomus</u> sp. (?)	9
	<u>Lactarius controversus</u> (Pers. ex Fr.) Fr.	6,10,16,19,49
	<u>Leccinum aurantiacum</u> (Bull.) S. F. Gray	6,15,16,40,50,51
	<u>L. durusculum</u> (Schulzer in Fr.) Sing.	53
	<u>Tricholoma irinum</u> (Fr.) Kumm.	18
<u>P. canescens</u> Smith	<u>C. geophilum</u>	6
Black x White hybrids (Aigeiros x Leuce)		
<u>P. nigra</u> L. x <u>alba</u>	<u>Glomus</u> sp. (?)	9
Black (Aigeiros)		
<u>P. deltoides</u> Bartr.	<u>C. geophilum</u>	48
	<u>Glomus</u> sp.	54,55
	<u>Lepista nuda</u> (Bull. ex Fr.) Cooke	10
	<u>Sclerocystis</u> sp. (?)	55
<u>P. x euramericana</u> (Dode) Guinier	<u>C. geophilum</u>	6
		6
[= <u>P. x canadensis</u> Moench]	<u>Gl. mosseae</u> (Nicol. & Gerd.) Gerd. & Trappe	38
[= <u>P. x robusta</u> Schn.]	<u>Hebeloma hiemale</u> Bres.	11
[= <u>P. x serotina</u> Hartig]	<u>Tuber albidum</u> Pico	12
	<u>T. borchii</u> Vitt.	22
	<u>T. magnatum</u> Pico	26

Table 4 continued

Poplar group (genus section (after Peace, 1952))		Literature reference (see Table 5)
Poplar species	Fungal species	
Black (Aigeiros) continued		
<u>P. x marilandica</u> Bosc. [= <u>P. nigra</u> x <u>euroamericana</u> .]	<u>Glomus</u> sp. (?)	9
<u>P. nigra</u> L.	<u>C. geophilum</u>	6,13
	<u>Glomus</u> sp. (?)	9
	<u>Inocybe boltoni</u> Heim var. <u>ionipes</u> Boud.	57
	<u>Lepista nuda</u>	10
<u>P. nigra</u> var. <u>italica</u> Du Roi	<u>Glomus</u> sp. (?)	4 <sup>a</sup> , 5 <sup>a</sup> , 6
Balsam x Black hybrids (Tacamahaca x Aigeiros)		
<u>P. x berolinensis</u> Dippel ( <u>P. laurifolia</u> Ledeb. x <u>nigra</u> )	<u>Glomus</u> sp. (?)	9
Balsam (Tacamahaca)		
<u>P. angustifolia</u> James	<u>C. geophilum</u>	48
<u>P. balsamifera</u> L. [= <u>P. tacamahaca</u> Mill.].]	<u>C. geophilum</u>	48
<u>P. trichocarpa</u> Torr. & Gray	<u>Amanita muscaria</u> (L. ex Fr.) Pers. ex Hooker	47
	<u>Boletus edulis</u> Bull. ex Fr.	47
	<u>C. geophilum</u>	47
	<u>Hebeloma crustuliniforme</u> (Bull. ex St. Am.) Quel.	47

<sup>a</sup>Tree species deduced from text, but subject to some doubt.



Table 4 continued

Poplar group (genus section (after Peace, 1952))		Literature reference (see Table 5)
Poplar species	Fungal species	
Aspens (Tremulae)		
<u>P. tremula</u> L.	<u>Cenococcum geophilum</u>	2,6,7,8,10,13,20,42,56
	<u>Clitopilus prunulus</u> (Scop. ex Fr.) Kumm.	1
	<u>Cortinarius cinnamomeus</u> (L. ex Fr.) Fr.	21
	<u>C. collinatus</u> (Pers. ex Fr.) Fr.	32,33,34
	<u>Glomus</u> sp. (?)	9
	<u>Gyromitra esculenta</u> Pers. ex Fr.	42
	<u>Lactarius contraversus</u>	17,52
	<u>L. volemus</u> (Fr.) Fr.	31
	<u>Leccinum aurantiacum</u>	1,3,19,24,27,28,30,36
		39,43,44,49,52,53
	<u>L. duriusculum</u>	1
	<u>L. scabrum</u>	24,36
	<u>Paxillus involutus</u>	1
	<u>Russula delica</u> Fr.	31
	<u>R. populea</u> Peyr. nom. nud.	15,52
	<u>R. virescens</u> (Schaeff. ex Zant.) Fr.	31,33
<u>P. tremuloides</u> Michx.	<u>C. geophilum</u>	48
	<u>Leccinum aurantiacum</u>	46
	<u>L. scabrum</u>	45
	<u>Pisolithus tinctorius</u> (Pers.) Coker & Couch	37

Table 5. The literature references for Tables 3 and 4<sup>a</sup>

Ref. no.	Author & Date	Ref. no.	Author & Date
1	Becker, 1956	15	Kalmar, 1950
2	Boullard & Dominik, 1958	16	Kalmar, 1954
3	Boullard & Moreau, 1962	17	Khokhryakov, 1956
4	Dangeard, 1896 <sup>b</sup>	18	Komirnaya & Fursayev, 1953
5	Dangeard, 1900 <sup>b</sup>	19	Lidl, 1939
6	Dominik, 1958 <sup>b</sup>	20	Lihnell, 1942
7	Dominik, 1961a	21	Masui, 1927
8	Dominik, 1961b	22	Mattirolo, 1934
9	Dominik & Ihnatowicz, 1979 <sup>b</sup>	23	Mattirolo, 1934 <sup>b</sup>
10	Fontana, 1961	24	Melin, 1923
11	Fontana, 1963 <sup>b</sup>	25	Melin, 1946
12	Fontana & Palenzona, 1969 <sup>b</sup>	26	Mosca, 1973 <sup>b</sup>
13	Frydman, 1957	27	Moser, 1958
14	Heim, 1957	28	Munch, 1927

<sup>a</sup>As cited in Trappe (1962) unless otherwise indicated in a footnote.

<sup>b</sup>See Literature Cited in this dissertation.

Table 5 continued<sup>a</sup>

Ref. no.	Author & Date	Ref. no.	Author & Date
29	Parrot, 1959	43	Smotlacha, 1912
30	Peyronel, 1920	44	Smotlacha, 1942
31	Peyronel, 1921	45	Trappe, 1957
32	Peyronel, 1922a	46	Trappe, 1960
33	Peyronel, 1922 <sup>b</sup>	47	Trappe, 1961
34	Peyronel, 1922b	48	Trappe, 1962
35	Rayner, 1935	49	Ulbrich, 1936
36	Schieferdecker, 1955	50	Vasil'kov, 1944
37	Schramm, 1966 <sup>c</sup>	51	Vasil'kov, 1954
38	Shuja et al., 1971 <sup>c</sup>	52	Vasil'kov, 1955
39	Shvartsman, 1958	53	Vasil'kov, 1956
40	Singer, 1949	54	Vozzo, 1969 <sup>c</sup>
41	Singer & Digilio, 1957	55	Vozzo & Hacskaylo, 1974 <sup>c</sup>
42	Siren & Bergeman, 1951	56	Wojciechowska, 1960
		57	Zerova, 1966 <sup>d</sup>

<sup>a</sup>References as cited in Trappe (1962) unless otherwise stated in a footnote.

<sup>b</sup>As reviewed in Kelley (1937).

<sup>c</sup>See Literature Cited in this dissertation.

<sup>d</sup>As reviewed in Review of Applied Mycology (1967) 46:23.

Table 6. Poplars surveyed for mycorrhizae, with their mycorrhizal status (+ = present; - = absent; blank = no data)

Genus section and poplar species <sup>a</sup>	ect <sup>b</sup>	ecten <sup>b</sup>	end <sup>b</sup>	aut <sup>b</sup>	Literature ref. <sup>c</sup>
Leuce					
<u>P. alba</u>	+		+	+	Dominik, Trappe
<u>P. canescens</u>	+				Trappe
Aigeiros x Leuce					
<u>P. nigra</u> x <u>alba</u>	-		+	-	Dominik
Aigeiros					
<u>P. deltoides</u>	+		+	+	Dominik, Trappe Vozzo & Hacskeylo
<u>P. x euramericana</u>	+	+	+	+	Dominik, Mejstrik, Trappe, V & H
<u>P. fremontii</u> S. Wats.	+		-		V & H
<u>P. x marilandica</u>	+		+	-	Dominik
<u>P. nigra</u>	+		+	-	Dominik, Trappe
<u>P. regenerata</u> Henry	+		-	-	Dominik
Tacamahaca x Aigeiros					
<u>P. x berolinensis</u>	+		+	-	Dominik
(Tacamahaca x Aigeiros) x Aigeiros					
<u>P. x generosa</u> Henry x <u>nigra</u>	+		+		Levisohn
Tacamahaca					
<u>P. angustifolia</u>	+				Trappe
<u>P. balsamifera</u>	+				Trappe
<u>P. candicans</u> Aiton	+		-	-	Dominik
<u>P. trichocarpa</u>	+				Trappe
Leucoides					
<u>P. heterophylla</u> L.	-		-	+	V & H
Tremulae					
<u>P. grandidentata</u> Michx	+		-		V & H
<u>P. tremula</u>	+	+	+	-	Dominik, Trappe, Melin
<u>P. tremuloides</u>	+		+		Trappe, V & H, Mc
Tremula x Aigeiros					
<u>P. tremula</u> x <u>P. x euramericana</u>	+		-	-	Dominik

<sup>a</sup>Includes varieties and subspecies.

<sup>b</sup>ect = ectomycorrhizal; ecten = ectendomycorrhizal; end = endomycorrhizal; aut = autotrophic.

<sup>c</sup>Dominik (1958), Levisohn (1957), McDougall & Jacobs (Mc) (1927), Mejstrik (1971), Melin (1923 as reviewed in Kelley (1937)), Trappe (1962), and Vozzo & Hacskeylo (V & H) (1974).

list, nor do they detail any of the ecology or physiology of the symbioses.

Ecology and physiology of mycorrhizal poplars Melin (1923 as reviewed in Kelley (1937)) studied aspen mycorrhizae and concluded that the symbiosis was mutualistic; anatomical investigations and pure culture experiments showing no injury to the tree and a distinct advantage in terms of growth to the fungus. He postulated that nutrient exchange takes place between the fungus and the plant which " ... long keeps both tissues active ...".

Dominik (1956, 1958) found that soil inoculation for establishment of mycorrhizae with poplars was "superfluous," since natural associations developed rapidly. He considered that endomycorrhizae formed only on 1- to 2-year-old plants, and that they were later replaced by ectomycorrhizae. His evidence, however, was not based on replicated experiments, but rather on limited observations. He also suggested that endomycorrhizae form on " ... extremely unnatural environments like wastes and rubble." This could have been due to a lack of ectomycorrhizal inoculum, the herbaceous plants always present in such situations having maintained the endomycorrhizal fungi. The trees would then have more chance of forming endomycorrhizae than ectomycorrhizae.

Overall, Dominik concluded that poplars can be mycotrophic or autotrophic, depending on the age of the plant and the availability of a suitable symbiont. The fact that they can survive autotrophically for long periods is of great advantage to them as pioneer

plants, and is consistent with their role in afforestation of " ... virgin areas after cataclysms and after the retreat of the glaciers." He did not draw any conclusions as to the benefit derived by the trees from the symbioses, except in the so-called "tolypophagic" type of infection. It is unclear exactly what this was, but it may have been caused by a weak pathogen, rather than a mycorrhizal fungus.

Mejstrik (1971) studied P. x euramericana on two spoil banks in Czechoslovakia. Both sites had high pH values of 7.1-7.7. Mean phosphorus values in the upper 50 cm of soil were 27 and 76 mgkg<sup>-1</sup> (expressed as citrate-soluble P<sub>2</sub>O<sub>5</sub>), two relatively low figures, indicating the need for good mycorrhizal establishment for satisfactory growth of most plants. The study was conducted over two years, and involved sampling roots at depths of 0-10 cm and 10-30 cm. Samples were taken in May, July, and September. Two parameters were measured: strength of mycorrhizal development as "weak", "medium", or "strong"; and frequency of mycorrhizae, indicated by "0", "+", "++", "+++", or "++++". The former was somewhat quantitative, being based on percentage of roots mycorrhizal. The latter was apparently a qualitative assessment, and was made only for ectomycorrhizae. Endomycorrhizae were simply recorded as present or absent. The age, time since planting, and condition of the trees were not reported, and it is unclear if transplants or naturally revegetated trees were involved. These studies revealed that all three types of mycorrhizae were present. Endomycorrhizae were most common in the upper 10 cm of soil, while the reverse was true for the other two types. Cenococcum geophilum was a common symbiont, consistent with

its somewhat pioneering habit and tolerance of extreme conditions (Trappe, 1977). Ectomycorrhizae were present at all sampling times, while ectendomycorrhizae did not occur in May, and were most abundant in September. The spectrum of different "taxa" of mycorrhiza was large, nine of Dominik's (1959) classes being found, although the only one that can be identified to fungal species was that caused by C. geophilum. In the site with the lowest phosphorus concentration, especially in September, the mycorrhizal spectrum was smallest, as were the strength of development and frequency figures. Mejstrik did not measure or comment upon the effects of mycorrhizal establishment on the plants.

In North America, Vozzo & Hacskeylo (1974) showed that endomycorrhizae of cottonwood respired at a higher rate than non-mycorrhizal roots. They considered this to be evidence of energy processes related to nutrient uptake, citing enhanced growth rate of mycorrhizal plants as evidence for increased requirement of nutrients. They presented, however, no evidence of such enhanced growth rate, and differences in respiration could have been simply due to the presence of different organisms with different rates of oxygen consumption. Differences in the biochemistry of mycorrhizal and non-mycorrhizal roots occurred. Phytosterol was found in autotrophic roots, but not in mycorrhizae. Arganine, asparagine, histidine, tannin, and oxidase occurred only in endomycorrhizae.

The only other recent study of poplar mycorrhizae in the U.S.A. also relates to spoil banks (Harris & Jurgensen, 1977). Each of

two sites in Michigan was planted with 2,000 hybrid poplar plants (species unnamed). One was an area of copper tailings, and the other an iron tailing site. Both sites were low in nitrogen, phosphorus, and potassium; both were slightly alkaline and had no organic matter. The first site was limited in water supply, while the latter tended to become saturated after heavy rainfall. An inoculum was applied, consisting of supernatant made by shaking soil from a willow site in water. Control cuttings were untreated. The roots were sampled at an unspecified time after planting ("during the summer") and investigated for mycorrhizae. No differences were noted between inoculated and uninoculated roots. All trees from the iron mine were mycorrhizal, whereas no plants on the copper tailings had mycobionts. Mean heights of the plants on the two sites were significantly different, and this was attributed to the differences in mycorrhizal development. This conclusion is untenable because of the many uncontrolled variables such as microclimate, metal concentration and type, pH, soil structure, and nutrient levels. A somewhat unusual procedure was used in this study. Roots of the non-mycorrhizal poplars were compared with roots of aseptically grown pines by scanning electron microscopy. Their similarity was presented as evidence for lack of mycotrophy in the poplars.

An overview      The literature contains evidence of the mycorrhizal status of 20 poplar species. All but one, P. heterophylla, have been shown to be mycorrhizal, the spectrum including the



three major types of mycotrophy, ectomycorrhizae, endomycorrhizae, and ectendomycorrhizae. Eight species are capable of autotrophic growth.

Of the 38 named fungal associates, 11 are known only from unnamed species in the genus Populus, the remaining 27 being associated with known species. Only four fungi have been proved mycorrhizal in pure culture synthesis, the remainder are assumed mycorrhizal from constant association with poplars, or from hyphal connections traced from sporocarps to mycorrhizae. The list includes fungi from 17 basidiomycete, 2 ascomycete, and 2 phycomycete genera (although one of the phycomycetes is subject to some doubt), and one imperfect genus (Genococcum). C. geophilum was mycorrhizal with all but two poplar species surveyed, the other fungi rarely being recorded from more than one.

The physiology and ecology of poplar mycorrhizae are largely uninvestigated. For P. deltoides, endomycorrhizae respire more than non-mycorrhizal roots, and have a different biochemistry. There is some evidence for mycorrhizal differences between and within sites, and some temporal differences have been shown, but no reliable information on effects of mycotrophy on growth or establishment of poplars exists. In 1950, Kelley wrote '... poplars and aspens ... have been studied, although not in much detail but their mycorrhizal character is established.' Except for information on mycobiont species, the situation has changed little since then.

## The Endogonaceae

Introduction     The Endogonaceae is a family of the order Mucorales in the class Zygomycetes (Webster, 1970). This family is of particular interest to a wide scientific community because of relationships some of its members have with plants. Many species form mycorrhizae, and as far as is known, all the phycomycetous (vesicular-arbuscular or arbuscular) endomycorrhizae are formed by fungi in the Endogonaceae. The genera Acaulospora and Gigaspora are not known to have any other habit, and most, if not all, of the species in Glomus are mycorrhizal.

Gerdemann & Trappe (1974) completely revised the family, extending Thaxter's (1922) concept of four genera, two of which were later combined by Zycha (1935 as cited in Gerdemann & Trappe (1974)), to one of seven genera. This revision was done by resurrecting two previously described genera (Glomus and Modicella), and by raising two new ones (Acaulospora and Gigaspora), species of which already had been placed in Endogone (Nicolson & Gerdemann, 1968) or described informally and worked on experimentally (Gerdemann, 1955; Gerdemann & Nicolson, 1963; Mosse, 1970a, 1970b, 1970c, 1972). Since that time, two more genera (Complexipes and Entrophospora) have been added (Ames & Schneider, 1979; Walker, 1979 and Part 3 herein).

The positioning of the family in the Zygomycetes is based on the type genus, Endogone, which forms zygospores arising from the

conjugation of two progametangia, or occasionally, from two suspensor cells (Gerdemann & Trappe, 1974; Thaxter, 1922). All the remaining genera are asexual, being divided into three groups; the chlamydo-sporic species (Complexipes, Glaziella, Glomus, and Sclerocystis), the azygosporic species (Acaulospora, Entrophospora, and Gigaspora), and the sporangial species (Modicella). They are placed together in the family because of their similarity of habit and form (i.e., production of sporocarps, hyphal similarities, presence of structural chitin), although, as Gerdemann & Trappe (1974) say there is no very good evidence to support this placing. The position in the Mucorales is based on even less evidence, i.e., the production of sporangia in one genus (Modicella). However, none of the other species form sporangia, and the only genus forming the sexual stage is Endogone. A revision of the family may be desirable. The study of the whole group is hampered by the failure of efforts to produce pure cultures from its members, except in a few cases which have not been repeated satisfactorily (Gerdemann & Trappe, 1974, 1975).

Taxonomic concepts      The taxonomy of the group is based on such morphological characters as size, color, shape and wall-structure of individual spores; and on size, color, shape, peridial development, glebal form, and positioning of spores in sporocarps, as well as the presence or absence of sporocarps (Gerdemann & Trappe, 1974). With the exception of Endogone, all the genera are asexual, and there is, therefore, no certainty that the species and genera

are truly different in the sense of fungi that can be traced through all their forms to the "perfect" state. However, the species can be maintained in pot culture, and inoculation with a particular type of spore results in the later production only of that type (Mosse, 1972). Indeed, the same species produce spores of indistinguishable morphology regardless of host plant, soil type, or climatic factors. Some species are recognized from all over the world, indicating that the morphological characteristics are constant, and that current taxonomic concepts are useful and effective.

Endogone Link (1809 as cited in Gerdemann & Trappe (1974))

Zygospores develop from the conjugation of two gametangia; with spores budding from the apex of the larger of two gametangia, from the point of the gametangial union, or from two separated suspensor cells (Figure 3). Spores are thick-walled, and have a continuous endospore formed after the outer wall is created by enlargement of the gametangial wall (Gerdemann & Trappe, 1974; Kanouse, 1936).

All known Endogone species are sporocarpic, although peridial and glebal development are variable. In some species, e.g., E. acrogena Gerdemann, Trappe & Hosford, no distinct peridium is formed, while in others, e.g., E. flammicorona Trappe & Gerdemann, there is a distinctly differentiated sporocarp wall. Similarly, some species have spores formed naked in the gleba, while others

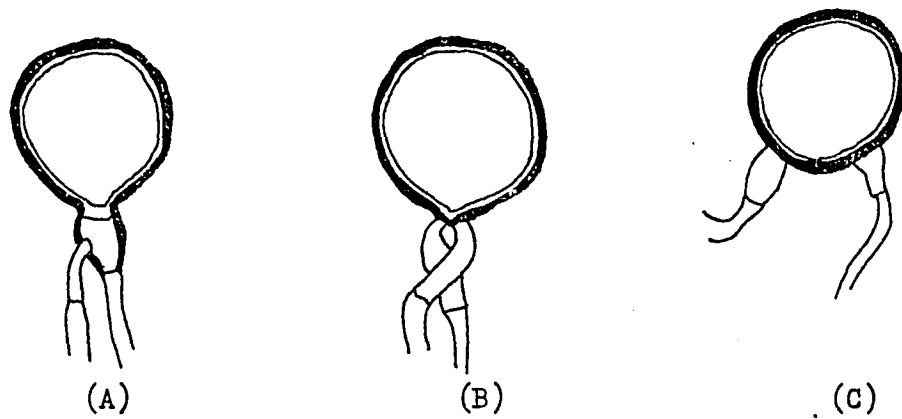


Figure 3. Formation of Endogone zygospores

- (A) Spore budding from the larger of two gametangia.
- (B) Spore formed from the point of gametangial union.
- (C) Spore formed between two suspensor cells.

(After Kanouse, 1936)

have distinctive hyphal cells forming a tight weft around individual spores.

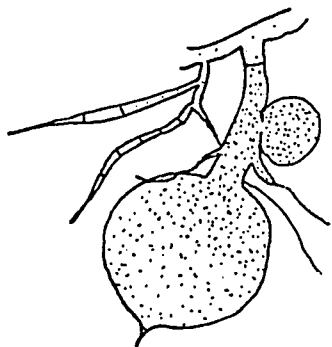
The only known mycorrhizal associations of Endogone species are for E. lactiflua Bk. & Br., which is ectomycorrhizal with pines (Fassi et al., 1969).

I have examined only a few specimens of E. lactiflua, E. flammicorona, and E. pisiformis Link ex Fries, and these not in great detail. In Iowa, I have found only a fragment of a sporocarp that might contain a few spores of E. lactiflua. Full descriptions of the species in Endogone can be found in Gerdemann & Trappe (1974), Tandy (1975), Thaxter (1922), and Warcup (1975).

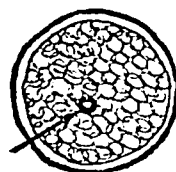
Acaulospora Gerdemann & Trappe (1974)      This genus has a most interesting mode of spore formation (Figure 4). At first, the somatic hypha begins to enlarge at the tip, forming peculiar wispy hyphae that protrude into the soil. The tip gradually enlarges to become a bulbous vesicle, from which the wispy hyphae still protrude. From the side of the vesicle, a spore begins to form, budding away from a collar-like structure. The contents of the mother vesicle empty into the spore as it enlarges, and as the walls thicken. Finally, the mother vesicle collapses, and the pore through which the contents passed becomes occluded. In some species, the mother vesicle becomes completely detached, leaving the sessile spore without any sign of its origin, except for the collar around the occluded pore. In other species, the vesicle,

Figure 4. The development of an azygospore of Acaulospora (After Gerdemann & Trappe, 1974)

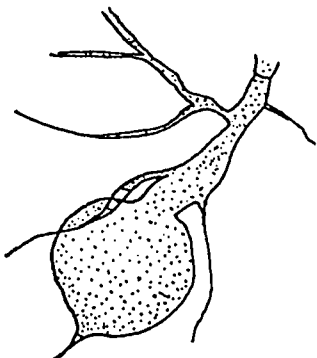
- (A) Somatic hypha begins to swell and wispy hyphae form.
- (B) The bulbous mother vesicle is formed.
- (C) The azygospore begins to bud off the neck of the mother vesicle.
- (D) The contents of the mother vesicle empty into the azygospore.
- (E) The pore through which the contents passed is occluded, and the mother vesicle collapses.
- (F) In many instances, the spore becomes completely detached. The collar around the occluded pore is often then evident (arrowed).



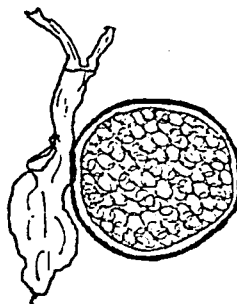
(C)



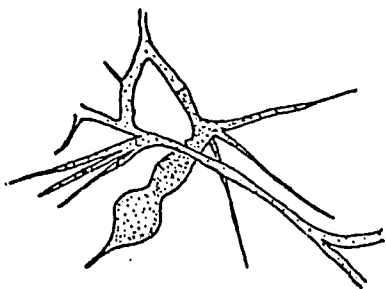
(F)



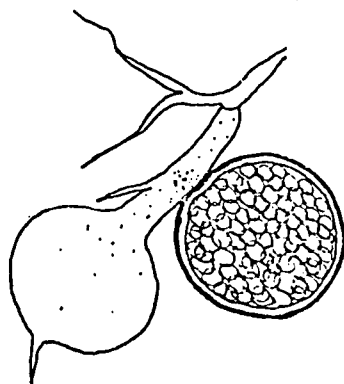
(B)



(E)



(A)



(D)



or remnants of it, usually remains attached to the spore, and can be seen on microscopic examination.

Spores of Acaulospora are considered to have some similarities with zygospores, because of the mother vesicle and attached hyphae. However, since no conjugation or other form of sexual fusion has been observed, they are known as "azygospores"; that is, "not-zygospores." Similar hyphae on the vesicle of Entrophospora, and on the suspensor cell of Gigaspora spores, lead to the use of the same term for spores of these two genera.

Speciation in Acaulospora is based on size, color, number of walls in the spore, and ornamentation of spore walls. There are presently six described species.

A. laevis Gerdemann & Trappe (1974) is a large-spored species, with spores 119-300 x 119-520  $\mu\text{m}$ . The outer wall is smooth, yellow-brown to red-brown, and there are two membranous inner walls, the innermost of which is sometimes minutely roughened. This species is the "honey coloured, sessile spore" of Mosse (1970a, 1970b, 1970c).

A. trappei Ames & Linderman (1976) is the smallest and duller species in the genus. It has globose to ellipsoid or obovoid spores 42-99 x 42-70  $\mu\text{m}$  in diameter. The spores were described originally as possessing only one wall, but specimens I have examined from pot cultures derived from the type collection have two; a thin outer wall that is minutely roughened, and a thinner membranous inner wall, which is difficult to see. The inner wall may develop with maturity, which may explain this difference if

the specimens used for the original description were not fully mature. Spores of A. trappei may form within the roots of plants. Such sporulation in roots has been observed for Gigaspora and Glomus species, but not for other species of Acaulospora.

A. gerdemannii Schenck & Nicolson (Nicolson & Schenck, 1979), known only from Florida, is a fairly large globose-spored species, with Azygospores of 200-250  $\mu\text{m}$  in diameter. The spore has a thick dull-brown outer wall, with highly convoluted cerebriform folds 10-12  $\mu\text{m}$  high on the outer surface. A single inner wall, which is hyaline and decorated with an alveolate reticulum, is present. The two walls readily separate when the spore is crushed.

A. scrobiculata Trappe (1977b) is a species with small, white to light brown azygospores. These spores have four walls, though only two are easily discernible. The inner walls are hyaline, and usually less than 1  $\mu\text{m}$  thick. The inside wall is minutely roughened, while the other two walls in the inner group are smooth. The outer wall is decorated by evenly spaced depressions 1-1.5 x 1-3  $\mu\text{m}$ , separated by ridges 2-4  $\mu\text{m}$  thick. This decoration causes the spores to look remarkably like miniature golf balls when examined through a compound microscope.

Two other Acaulospora species have been named. A. elegans Trappe & Gerdemann (Gerdemann & Trappe, 1974) has globose to reniform dull dark brown spores, 140-285 x 145-330  $\mu\text{m}$ . The outer wall is ornamented by densely crowded spines about 2  $\mu\text{m}$  high and 0.5  $\mu\text{m}$  in diameter. Over the top of this layer of spines is laid

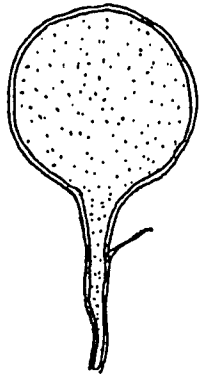
an alveolate reticulum  $5-6 \times 1 \mu\text{m}$ . This outer wall and reticulum enclose three hyaline inner walls totalling  $15 \mu\text{m}$  or less in thickness. A. bireticulata Rothwell & Trappe (1979) is similar in many ways to A. elegans. A. bireticulata has globose spores,  $150-155 \mu\text{m}$  in diameter, with the outer wall decorated by a polygonal reticulum,  $6-18 \mu\text{m}$  across. The ridges of the reticulum are  $2 \times 1.5-2 \mu\text{m}$  in dimensions. The reticulum overlays round-tipped processes on the spore surface. There are two inner hyaline layers, each about  $1 \mu\text{m}$  thick. The outer wall, also about  $1 \mu\text{m}$  thick, is dark grayish-green to grayish-brown.

All known Acaulospora species form vesicular-arbuscular mycorrhizae, some with characteristic lobed vesicles. A. laevis, A. scrobiculata, and A. trappei are known from my collections in Iowa, as well as one new species, A. spinosa sp. ined. (Part 4 herein).

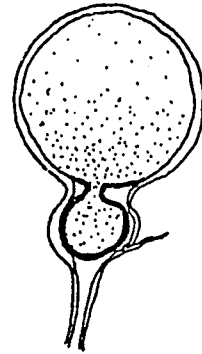
Entrophospora Ames & Schneider (1979) Entrophospora is a monospecific genus having many similarities with Acaulospora. Unlike Acaulospora, however, azygospores of Entrophospora are produced in the neck of the mother vesicle, rather than on its side (Figure 5). E. infrequens (Hall) Ames & Schneider has globose to ellipsoid spores,  $69-225 \times 69-164 \mu\text{m}$  in size. It has, in common with some of the Acaulospora species, a highly ornamented outer spore surface. The outer wall is covered by densely crowded hollow projections, giving it a superficial resemblance to A. spinosa.

Figure 5. The development of an Entrophospora azygospore  
(Drawn from the plates in Ames & Schneider, 1979)

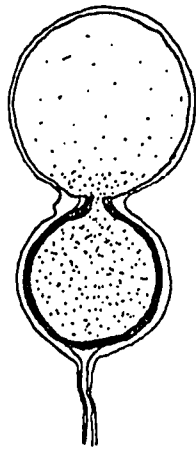
- (A) A bulbous mother vesicle forms.
- (B) The azygospore begins to develop in the neck of the mother vesicle.
- (C) The contents of the mother spore pass into the azygospore. Note the short protruding neck of the spore.
- (D) The spore contents are sealed off by plugging of the short neck. The mother vesicle collapses. Note the remnants of the neck of the mother vesicle surrounding the spore.



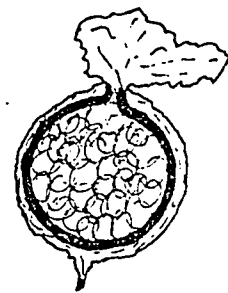
(A)



(B)



(C)



(D)

The outer wall is covered by the tightly adherent remnants of the neck of the mother vesicle. A thin separable membranous inner wall encloses the spore contents. Spores broken away from the mother vesicle can be confused with species of Glomus, since the point where the spore contents pass from mother vesicle to spore is thickened and takes the form of a short neck. Indeed, Hall (1977) originally named this species Gl. infrequens, because he had not found its developmental stages, and had only a few mature spores to examine.

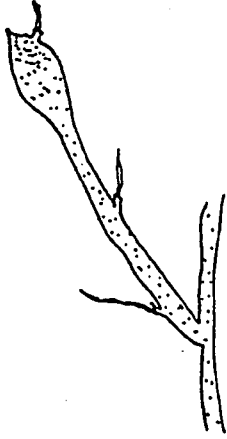
Attempts to obtain mycorrhizal formation with E. infrequens have failed to date, although it sporulates in abundance in company with other members of the family. The biology of this species needs elucidation. E. infrequens is common in Iowa, always accompanying mycorrhizal members of the Endogonaceae.

Gigaspora Gerdemann & Trappe (1974)      This genus has 11 described species, all with azygospores formed terminally on inflated hyphal cells (Figure 6). These bulbous cells are suspensor-like (Gerdemann & Trappe, 1974), and usually have a small peg-like process projecting towards the spore, from which extends a thin wispy hypha that appears collapsed and empty. However, this structure seems not to be gametangial in nature, and similar peg-like processes often are observed along the sporogenic hyphae.

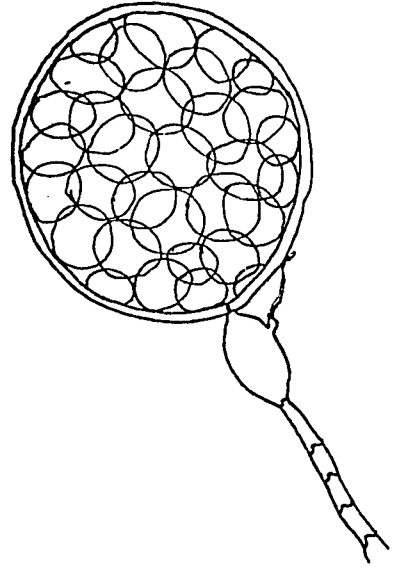
Species in this genus have accessory vesicles, sometimes known as "extra matrical vesicles," that form in soil on the somatic

Figure 6. Development of a Gigaspora azygospore

- (A) A hyphal tip begins to swell.
- (B) The swollen hypha takes on a bulbous appearance.
- (C) The azygospore is produced at the tip of the suspensor-like cell.
- (D) The spore is fully developed. The subtending hypha becomes septate, and the hypha and bulbous cell become empty.



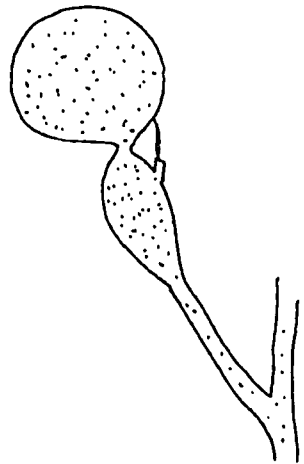
(B)



(D)



(A)



(C)



hyphae (Figure 7). These vesicles vary in size, shape, form, and color, and are used as taxonomic characteristics, though, fortunately, species normally can be identified without them. The extra-matrical vesicles usually are not found in soil sievings, except from pot cultures, because of their small size and delicate nature. The purpose, if any, that these structures serve is unknown, though Gerdemann & Trappe (1974) suggested that they may be temporary food storage organs, or modified subsporangial vesicles.

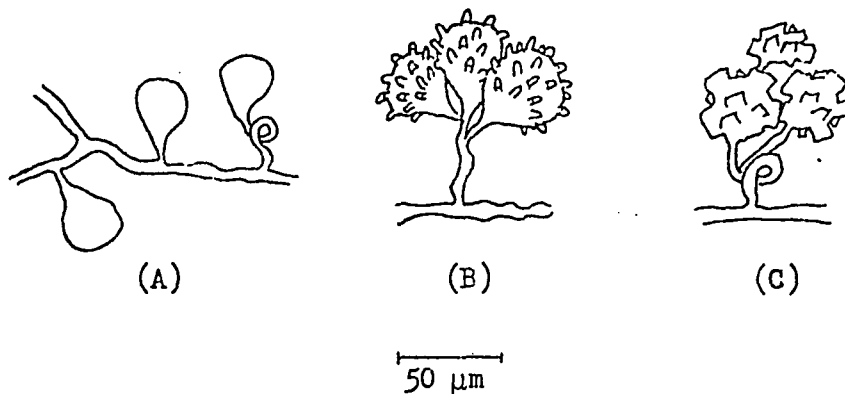


Figure 7. Extra-matrical vesicles of some Gigaspora species

- (A) Gigaspora calospora.
- (B) Gigaspora gigantea.
- (C) Gigaspora gilmorei.

Wall structure is a particularly useful taxonomic character in Gigaspora and Glomus. Three types of wall are recognized: (1) the one-layered wall, (2) the laminated wall, and (3) the membranous wall (Figure 8).

The one-layered wall is clearly distinguishable and invariant (except in thickness) among specimens of any particular taxon. Often, such a wall will separate from other walls when a spore is crushed. In species where such walls are tightly adherent and not readily separable, the walls are distinguished by color or structure (Figure 8A). Many species have an extremely thin, tightly adhering wall that may disappear as the spore matures.

The laminated wall is recognized as a separate wall, but is constructed of tightly fused layers. The layers are evident because of slight differences in refringence. Laminated walls begin as a single layer and become thicker with age by deposition of additional laminae (Figure 8B). The number of laminae therefore will vary from specimen to specimen, depending on the age and physiology of the spore. Sometimes laminae are extremely difficult to see. Applications of stains (e.g., cotton blue) and reagents (e.g., Melzer's reagent) may increase the ease of observation.

The membranous wall is found enclosing either other similar walls or the spore contents. The term "membrane" is not meant to imply the physiologist's cellular or subcellular membrane, but is used in the older and broader sense, to indicate a thin, more-or-less elastic covering. Whereas one-layered walls and laminated walls

Figure 8. Different kinds of walls in the Endogonaceae

(A) One-layered walls.

Two walls of approximately equal thickness have separated on crushing (left).

Two tightly adherent unequal walls have not separated on crushing, but are distinguishable structurally, and are constant among specimens (right).

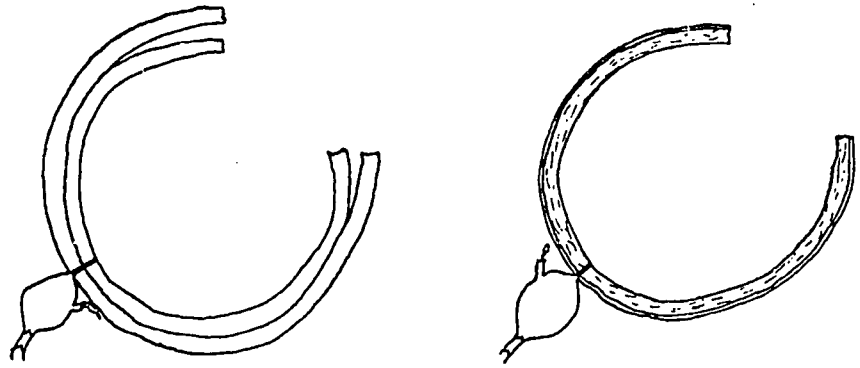
(B) Laminated walls.

Segments showing three different stages in the development of a wall with eight laminae.

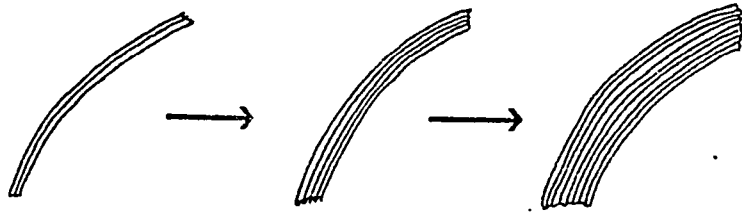
(C) Membranous walls (arrowed).

In a fresh, crushed specimen (left), the brittle one-layered outer wall has broken, but the inner membrane remains intact.

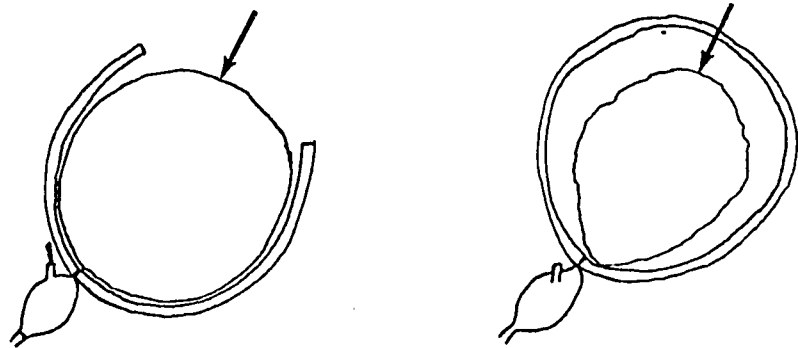
In a preserved specimen (right), the membrane has shrunk as the spore contents became plasmolysed.



(A)



(B)



(c)

are rigid, and break readily upon crushing, the membranous wall is somewhat elastic, and is fractured less easily. Unlike the other two wall types, these membranes usually shrink as the spore contents plasmolyse in mounting media (Figure 8C).

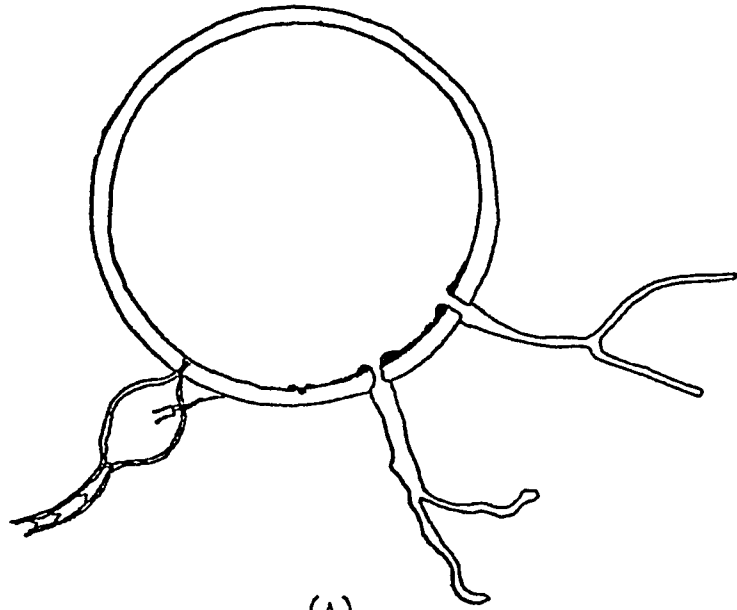
The wall structures used in taxonomy are those relatively easily observed by light microscopy. The actual structure probably is more complex, with many more layers at the ultrastructural level than are used in taxonomy (Mosse, 1970c).

The species of Gigaspora can be divided into two groups by mode of germination (Figure 9). One group germinates by direct regrowth through a relatively simple spore wall (Figure 9A). The other species have a more complicated wall structure, with inner membranes. Prior to germination, a compartment is formed by separation of one or more of these membranes from the outer walls. Extra structural material is deposited on the compartment walls, and radial walls develop, dividing the compartment into sub-compartments (Figure 9B). The germ tubes originate from these sub-compartments. Spores from at least one Acaulospora species germinate by compartmentalization (Mosse, 1970a, 1970c). Gigaspora spores are multinucleate, and more than one germ tube may be produced from a single spore. I have observed as many as five germ tubes from spores of Gi. rosea Nicolson & Schenck.

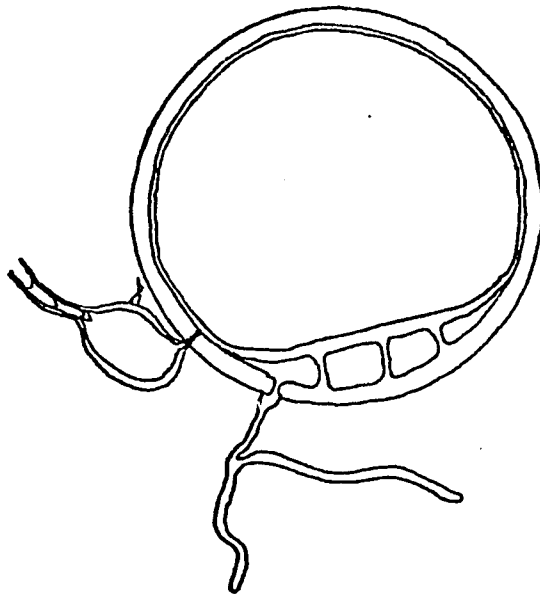
Figure 9. Germination modes of Gigaspora azygospores

(A) Germination directly through the spore wall without compartmentalization, e.g., Gi. rosea.

(B) Germination by compartmentalization. The compartments are formed by the inner membranous wall separating from the outer wall layer or layers, and extra material being laid down to thicken the membrane and form sub-compartments, e.g., Gi. heterogama.



(A)



(B)

Gi. aurigloba Hall (1977) is known only from New Zealand.

Spores are usually globose, 200-420 x 130-420  $\mu\text{m}$ , with occasional specimens reaching 520 x 520  $\mu\text{m}$ . The spores have a 2-4 layered wall structure, consisting of a single, pale to dull yellow outer wall, 6-16  $\mu\text{m}$  thick, and inner membranous walls, each approximately 1  $\mu\text{m}$  thick. Vesicles in the soil are echinulate to knobby, as much as 100  $\mu\text{m}$  in diameter, and borne in loose clusters, sometimes on coiled hyphae. Germination is by compartmentalization.

Gi. calospora (Nicolson & Gerdemann, 1968) Gerdemann & Trappe also germinates by compartmentalization. The spores are 150-285 x 165-412  $\mu\text{m}$ , globose to ellipsoid or oblong. The walls are double, with a rigid wall enclosing a thinner inner membrane. Vesicles are 23-33  $\mu\text{m}$  in diameter, and are single, and smooth or knobby. The spore is from hyaline to shiny brown, the complete color range being found in field collections in Iowa. This description is slightly different from the one originally published, but is based on examination of the type collection, kindly loaned by the Farlow Herbarium, and on numerous specimens from field collections.

Gi. coralloidea Trappe, Gerdemann & Ho (Gerdemann & Trappe, 1974) is named because its vesicles, which are 25-30 x 30-40  $\mu\text{m}$ , and brownish-yellow, form coralloid structures by growth of irregular, often forked projections, 5-15 x 4-7  $\mu\text{m}$  in size. These vesicles often are intermingled with thin-walled inflated projections, up to 10  $\mu\text{m}$  across. The spores are dark brown, with a single outer layer, 8-15  $\mu\text{m}$  thick, enclosing a hyaline membrane, 1-2  $\mu\text{m}$  in thickness.



The outer wall is decorated by irregularly-shaped hyaline warts and ridges, which often radiate out from the base of the spore. Germination mode is not recorded for this species.

Gi. gigantea (Nicolson & Gerdemann, 1968) Gerdemann & Trappe (1974) has greenish-yellow globose to ellipsoid spores, 353-368 x 345-398  $\mu\text{m}$  in diameter. It has an inner wall, 5-7  $\mu\text{m}$  thick, which is tightly covered by another, very thin, hyaline outer wall. The vesicles are echinulate to papillate. Gi. gigantea germinates by direct growth through the spore wall.

Gi. gilmorei Trappe & Gerdemann (Gerdemann & Trappe, 1974) possesses globose to subglobose (occasionally ellipsoid) hyaline spores, 204-320  $\mu\text{m}$  in diameter. The spore walls readily separate into two groups. The outer group is brittle, and 11  $\mu\text{m}$  in maximum thickness, consisting of a thin outer layer no more than 1  $\mu\text{m}$  thick, and a thick inner layer. The inner group of walls is flexible, as much as 7.4  $\mu\text{m}$  thick, and is made up of three membranous layers, the outer of which is less than 1  $\mu\text{m}$  thick. Gi. gilmorei has light brown suspensor cells, and clusters of knobby, hyaline to light brown vesicles, 15-25  $\mu\text{m}$  in diameter. The spores germinate by compartmentalization.

Gi. gregaria Schenck & Nicolson (Nicolson & Schenck, 1979) is similar to Gi. coralloidea, except in two characteristics: vesicles are clustered (3-16 per cluster), and knobby or tuberculate, rather than single and coralloid; and the outer wall-group of Gi. gregaria is double, consisting of two tightly-fused walls,

whereas the outer wall of Gi. coralloidea is single. Spores are globose, red-brown to dark-brown, and 250-448  $\mu\text{m}$  in diameter. They have irregular-shaped projections on the outer wall. Vesicles arise from coiled or twisted light brown hyphae, and are 14-31 x 22-48  $\mu\text{m}$ . Their tuberculate projections are 0.5-2.4 x 1.2-4.8  $\mu\text{m}$ . This species is so far known only from Florida. There is no information on its mode of germination.

Another brown-spored species is Gi. heterogama (Nicolson & Gerdemann, 1968) Gerdemann & Trappe (1974). This was one of the first Gigaspora species to be described, along with Gi. calospora and Gi. gigantea. Vesicles of Gi. heterogama are brown and clustered, 1-10 in a group, and formed on tightly-coiled hyphae. These vesicles are smooth to knobby, and 16-27 x 20-31  $\mu\text{m}$  in size. The light brown azygospores are usually spherical, 150-202  $\mu\text{m}$  in diameter, and have minute projections 0.5-2  $\mu\text{m}$  high covering the outer wall. This outer wall consists of two tightly-fused brittle layers, totalling 3-5  $\mu\text{m}$  in thickness, enclosing a membranous inner wall, 3-5  $\mu\text{m}$  thick.

One of the most striking species in the genus is Gi. margarita Becker & Hall (1976). The spores of this species look, as the specific name implies, like small pearls. They are globose, opaque white, and 260-480  $\mu\text{m}$  in diameter. The spore wall is 5-24  $\mu\text{m}$  thick, smooth and shiny, and composed of 4-10 laminations, the number increasing with age. The suspensor-like cell is hyaline to light brown. The vesicles form in tight clusters of 1-20 on

coiled hyphae, each vesicle being 22-32  $\mu\text{m}$  in diameter, and having a warty appearance. Both spores and vesicles turn brown with age. Gi. margarita germinates by direct growth of the germ tube through the spore wall.

At the other end of the color spectrum is Gi. nigra J. F. Redhead (Nicolson & Schenck, 1979). This species has large globose spores, 297-1000  $\mu\text{m}$  in diameter (mean 400  $\mu\text{m}$ ). The outer wall is black to dark brown, and is pitted with pores 7-10  $\mu\text{m}$  in diameter, giving the surface a reticulate appearance. These pores overlay smaller pores that consist of a series of coils, the structure of which is difficult to observe because of the opaque nature of the outer layer. A light brown, laminated inner wall is present. The suspensor-like cell often is attached laterally, and also is dark brown. Vesicles are smooth to knobby, 21-36  $\mu\text{m}$  in diameter, and dark brown, usually occurring in clusters of 3-12. Germination is by compartmentalization. Gi. nigra is known from Florida and Nigeria (Nicolson & Schenck, 1979), and from Texas (Ruth Ann Taber, Texas A. & M. University (1979); pers. comm.).

Gi. pellucida Nicolson & Schenck (1979) is very similar to Gi. gilmorei, though the spores, on average, are slightly smaller (58-212  $\mu\text{m}$  for Gi. pellucida compared with 204-320  $\mu\text{m}$  for Gi. gilmorei). However, Gi. pellucida spores may be irregularly shaped, 183-328  $\mu\text{m}$  or less, thus overlapping the range of Gi. gilmorei. The spore wall is separable into two units. The outer wall is brittle, 3-8  $\mu\text{m}$  thick, and formed of 2-3 laminae. The

inner wall is pliable, 1.5-5  $\mu\text{m}$  thick, and of a single layer. The inner wall of Gi. gilmorei is formed from four membranes. Vesicles are brown, 19-38  $\mu\text{m}$  wide, formed in clusters of 1-14 on coiled hyphae, and possess knobby projections about 5 x 10  $\mu\text{m}$ . The spores germinate by compartmentalization.

The last of the species in this genus is Gi. rosea Nicolson & Schenck (1979). This species bears considerable resemblance to Gi. margarita. The spores initially are opaque and pearly white. As the spores mature, a pink to peach-colored tint develops from the base. Eventually, the spore contents become clear, and the tint on the wall may cover more than half the spore. Spores mostly are globose, 230-305  $\mu\text{m}$  in diameter, and have a single, laminated wall of 2-5 layers that total 7.5  $\mu\text{m}$  or less in thickness. Vesicles are echinulate to papillate, 19-32  $\mu\text{m}$  wide, and in clusters of 5-12 on coiled hyphae. Germination is directly through the spore wall without compartmentalization. Collections from Iowa have walls with 1-7 laminae, and often have a tightly adherent hyaline outer wall, less than 1  $\mu\text{m}$  thick.

All species of Gigaspora form endomycorrhizae with arbuscules and hyphal coils, but without vesicles. Furlan & Fortin (1973) found vesicles in roots of onions mycorrhizal with Gi. calospora, but the photograph in their publication indicates a structure similar to extra-matrical vesicles, rather than the storage vesicles of a typical endomycorrhiza. In this instance, the mycorrhizal vesicles probably could be considered as aberrations,

since there are no further reports of such structures in mycorrhizae formed by Gigaspora species.

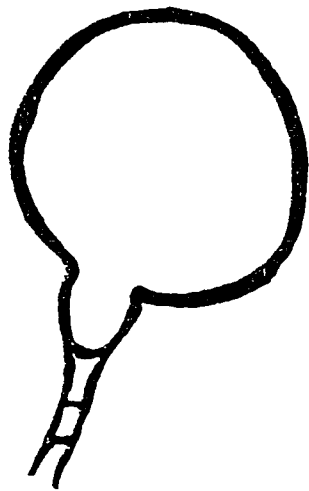
I have examined specimens of all species of Gigaspora except Gi. aurigloba. Except for Gi. heterogama, the specimens examined have included either the type collection or spores from pot cultures derived from the type collection. Gi. calospora, Gi. gigantea, Gi. gilmorei, and Gi. rosea are known from my collections in Iowa.

Glomus Tulasne & Tulasne (1845 as cited in Gerdemann & Trappe (1974)) This is the largest genus in the family, with 30 currently accepted species and one variety. Members of the genus produce chlamydospores in sporocarps, in loose clusters, or singly. Spores are usually terminal on undifferentiated hyphae (Figure 10), but may be intercallary. In most species, intercalary spores are uncommon, but in Gl. multicaulis Gerdemann & Bakshi, spores are formed predominantly in that fashion (Gerdemann & Bakshi, 1976). Glomus spores may be produced in soil, within roots, or, less commonly, on the soil surface or on ground vegetation, depending on species and conditions.

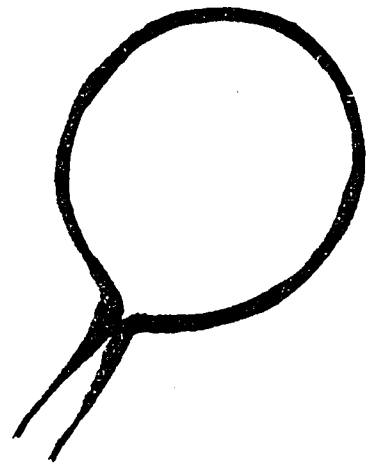
Germination of Glomus spores is usually by regrowth through the subtending hypha (Gerdemann & Trappe, 1974), but at least one species (Gl. pallidus Hall, 1977) germinates directly through the spore wall. Subgenera may be erected in the future delimited on germination characteristics for both Glomus and Gigaspora.

Figure 10. Typical chlamydospores of Glomus species

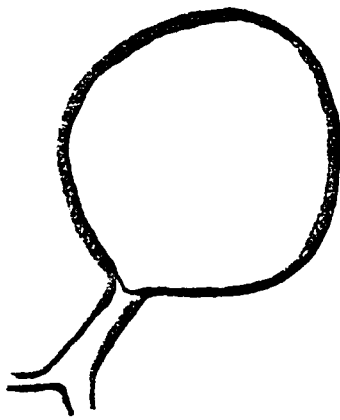
- (A) Spore with a funnel-shaped base. Spore contents occluded by thick septa  
e.g., Gl. mosseae.
- (B) Spore with a straight subtending hypha. Contents occluded by spore wall thickening  
e.g., Gl. fasciculatus.
- (C) Spore with contents separated from the hypha by a thin septum  
e.g., Gl. caledonius (Nicol. & Gerd.) Trappe & Gerdemann.
- (D) Spore with a constricted, recurved subtending hypha  
e.g., Gl. constrictus.
- (E) Intercalary spore  
e.g., Gl. multicaulis.



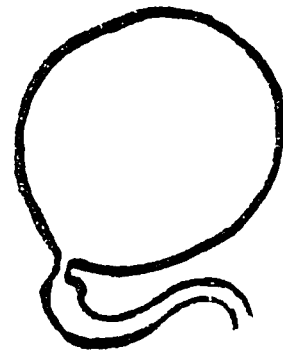
(A)



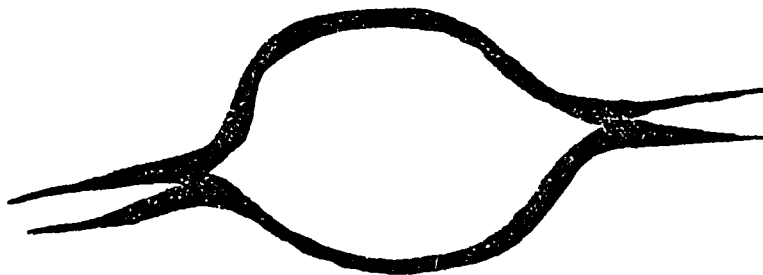
(B)



(C)



(D)



(E)

Another taxonomic characteristic used in the Endogonaceae is the "outer coat" (Figure 11). This is particularly common in Glomus, and consists of a hyaline to subhyaline amorphous layer that appears to be secreted onto the outer wall. The thickness of this coat varies with the developmental stage of the spore. In some species, the coat is present in early stages, and is sloughed later. In other taxa, the coat develops as spores mature, and is persistent at maturity, sometimes developing rounded bumps, possibly resulting from bacterial action (Gerdemann & Trappe, 1974). Some species (e.g., Gl. gerdemannii Rose, Daniels & Trappe, 1979) may have different numbers of walls at different growth stages. To avoid confusion, and for definite identification, a large series, preferably from a well-established pot culture, should be studied.

Only the eight species of Glomus known from Iowa will be described here. Details of described species in the genus are found in Becker & Gerdemann (1977), Daniels & Trappe (1979), Gerdemann & Bakshi (1976), Gerdemann & Trappe (1974), Hall (1977), Nicolson & Schenck (1979), Rose et al. (1979), Tandy (1975), and Trappe (1977b).

Gl. constrictus Trappe (1977b) has spores formed singly or in loose clusters in soil. The spores are 150-330  $\mu\text{m}$  in diameter, globose to subglobose, and dark brown to black in color, becoming shiny black at maturity. Spore walls are 2-layered, the outer layer often flaking off with maturity. The subtending hypha is recurved, and is characteristically inflated distad of the point



Figure 11. Detail of an endogonaceous spore (Glomus occultus sp. ined.) with an "outer coat". The coat remains intact on the left, but is sloughing on the right. Older spores may show no signs of the coat



of attachment, and is sharply constricted at the spore base, to give a superficial resemblance to a bulbous suspensor-like cell. The spore contents are sealed off by thickening of the wall at the base. This species can be confused with Gl. macrocarpus var. geosporus (Nicolson & Gerdemann) Gerdemann & Trappe. The latter does not, however, have the constricted, recurved hypha, and tends to be less shiny, and more red-brown than black. Trappe (1977b) discussed the differences between the two species.

Gl. epigaeus Daniels & Trappe (1979) forms spores in epigeous sporocarps, or hypogeously, either singly, or in loose clusters. Sporocarps are 2-8 x 3-15 mm, and can contain thousands of spores. There is no peridium, but the spores are produced in masses on a sterile basal pad of hyphae. Individual chlamydospores are globose to subglobose, (60-)75-140(-165) x 65-140  $\mu\text{m}$ . Spores are smooth and white when young, becoming yellow-brown to light-brown with maturity. The walls are composed of a subhyaline outer coat, 0.5-1  $\mu\text{m}$  thick, and an unlaminate single wall, not exceeding 10  $\mu\text{m}$  in thickness. The subtending hypha is thin-walled, and usually only 4-6  $\mu\text{m}$  in diameter. In many specimens, this hypha is attached so that it appears to be inserted into the spore wall. The spore contents are closed off by thickening of the inner portion of the spore wall (Figure 12).

Specimens of Gl. epigaeus found in Iowa are somewhat larger (up to 200  $\mu\text{m}$  in diameter), and have a deeper color, generally red-brown. They have not been found epigeously, or in sporocarps.

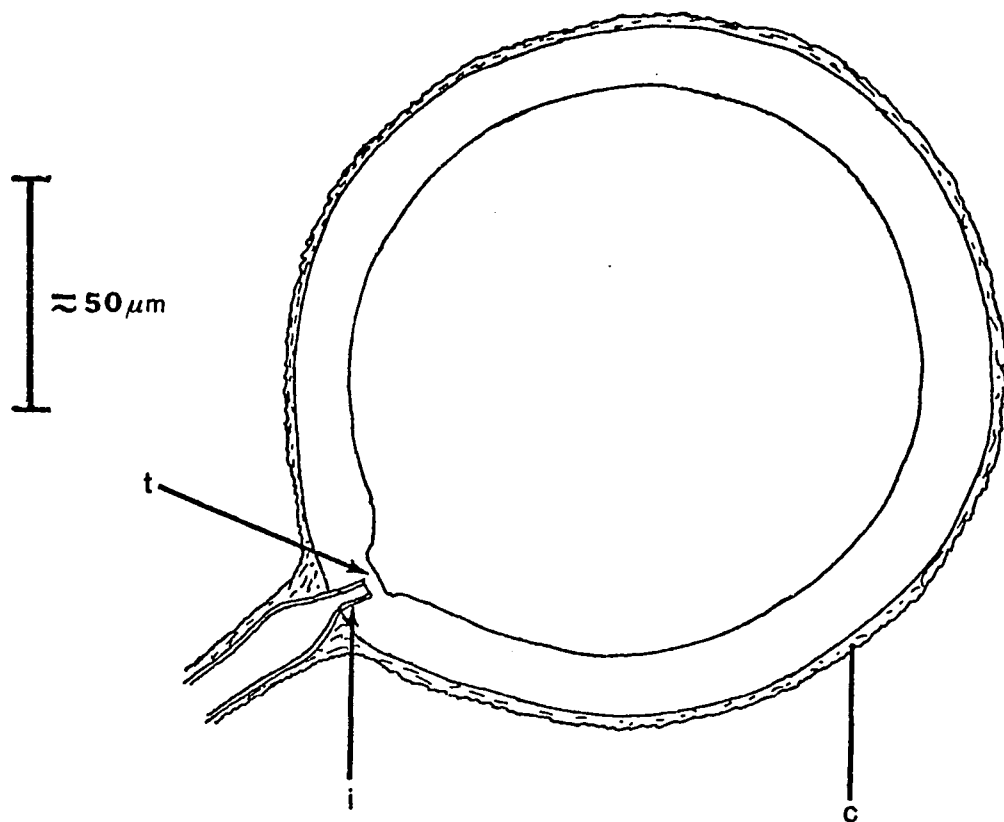


Figure 12. Chlamydospore of Glomus epigaeus.  
Note the apparent insertion of the subtending hypha  
into the spore wall (i), the septum-like wall-  
thickening occluding the spore contents (t), and  
the roughened outer coat (c).

However, the differences seem not to be great enough for creation of a new taxon. This species is known from greenhouses in Oregon and California, and from forests in Mexico. The specimens from Iowa constitute the first field record of Gl. epigaeus outside Mexico.

Gl. etunicatus Becker & Gerdemann (1977) has globose to subglobose spores, 68-144(-162)  $\mu\text{m}$  in diameter, which are formed singly either in soil or in dead roots. This species is characterized by an ephemeral hyaline outer coat that reaches a maximum thickness of 5  $\mu\text{m}$ , and sloughs as the spore matures. This coat covers a laminated wall, 2-8  $\mu\text{m}$  thick, and is rarely present on mature spores, necessitating a good series of specimens for positive identification. The subtending hypha is generally thin-walled, with thickening usually extending less than 30  $\mu\text{m}$  from the spore base. The spore often breaks from the hypha, making the point of attachment difficult to observe. A thin, curved septum, that later is obscured by spore wall thickening, separates the spore contents from the hypha. The spores are yellow-brown to brown, and, at maturity when the outer coat is lacking, are easily mistaken for Gl. macrocarpus spores.

The group of species that includes Gl. fasciculatus (Thaxter sensu Gerdemann) Gerdemann & Trappe (1974) is not delineated clearly. Gerdemann & Trappe (1974) believed that a more intensive study of the taxon was required, and their description was offered in a broad sense. My own initial studies lead me to

the conclusion that this is a group of similar species, rather than one taxon. This view is supported by personal communications with other workers, and I plan further studies on the group. The description given by Gerdemann & Trappe (1974) follows:

"Chlamydospores borne free in soil, in dead rootlets, in loose aggregations, in small compact clusters, and in sporocarps. Sporocarps up to 8 x 5 x 5 mm, irregularly globose or flattened, tuberculate, grayish brown. Peridium absent. Chlamydospores 35-105  $\mu$  when globose, 75-150 x 35-100  $\mu$  when subglobose to obovate, ellipsoid, sublenticular, cylindrical, or irregular; smooth or seeming roughened from adherent debris. Spore walls highly variable in thickness (3-17  $\mu$ ), hyaline to light yellow or yellow-brown, the thicker walls often minutely perforate with thickened inward projections. Hyphal attachments 4-15  $\mu$  diam, occluded at maturity. Walls of attached hyphae often thickened to 1-4  $\mu$ m near the spore [p. 51]."

Species fitting this description would be better considered as members of the Gl. fasciculatus group, rather than as Gl. fasciculatus sensu stricta.

Gl. macrocarpus var. geosporus (Nicolson & Gerdemann, 1968)

Gerdemann & Trappe (1974) is described and raised to species status in Part 5 herein.

Gl. macrocarpus Tulasne & Tulasne (1845 as cited in Gerdemann & Trappe (1974)) var. macrocarpus Gerdemann & Trappe (1974) produces hypogeous chlamydospores borne singly, in loose clusters, or in sporocarps that include particles of the substrate. The sporocarps can be 12 mm wide and usually are irregular in shape. There is sometimes a white cottony peridium which collapses upon handling.

Often, spores protrude to the surface of the peridium. Individual chlamydospores are 93-206(-230)  $\mu\text{m}$  in diameter, normally globose, though sometimes ellipsoid to obovoid because of mutual pressure in the sporocarp. The spore wall is laminated and smooth, though it may appear roughened from adherent debris. Spores are light yellow-brown to brown, with a thick-walled, persistent subtending hypha, usually longer than 30  $\mu\text{m}$ . The wall occludes the pore at the base by thickening that extends 10-30  $\mu\text{m}$  along the subtending hypha.

Gl. microcarpus Tulasne & Tulasne (1845 as cited in Gerdemann & Trappe (1974)) is easily recognizable by the small spores (25 x 25 - 55 x 32  $\mu\text{m}$ ) which are highly variable in shape. Spores are hyaline to yellow-brown, with a laminated wall not exceeding 7  $\mu\text{m}$  thick. As with Gl. macrocarpus var. macrocarpus, the surface is smooth, but may appear roughened because of adherent debris. Spores may be produced in sporocarps with or without a peridium, or singly and in loose clusters in the soil. The subtending hypha is almost occluded by wall thickening at maturity.

The last of the common species of the genus is Gl. mosseae (Nicolson & Gerdemann, 1968) Gerdemann & Trappe (1974). This species produces beautiful yellow spores with a distinctive funnel-shaped base. It is the "yellow vacuolate" spore of Mosse & Bowen (1968a), but not the "yellow vacuolate" spore of Redhead (1977). Spores of Gl. mosseae are produced either singly in soil, or in small characteristic hypogeous sporocarps containing 1-32 spores

(usually 2-6). Sporocarps may reach 1 mm in diameter, and are globose to subglobose, with a variable peridium of irregularly-branched anastomosing hyphae. Individual spores are globose to ovoid, 60-320  $\mu$ m in diameter, and with a double wall. This double wall consists of a very thin outer layer, rather difficult to distinguish, and an inner wall, 2-7  $\mu$ m thick.

All species of Glomus so far found in Iowa are known to produce vesicular-arbuscular mycorrhizae.

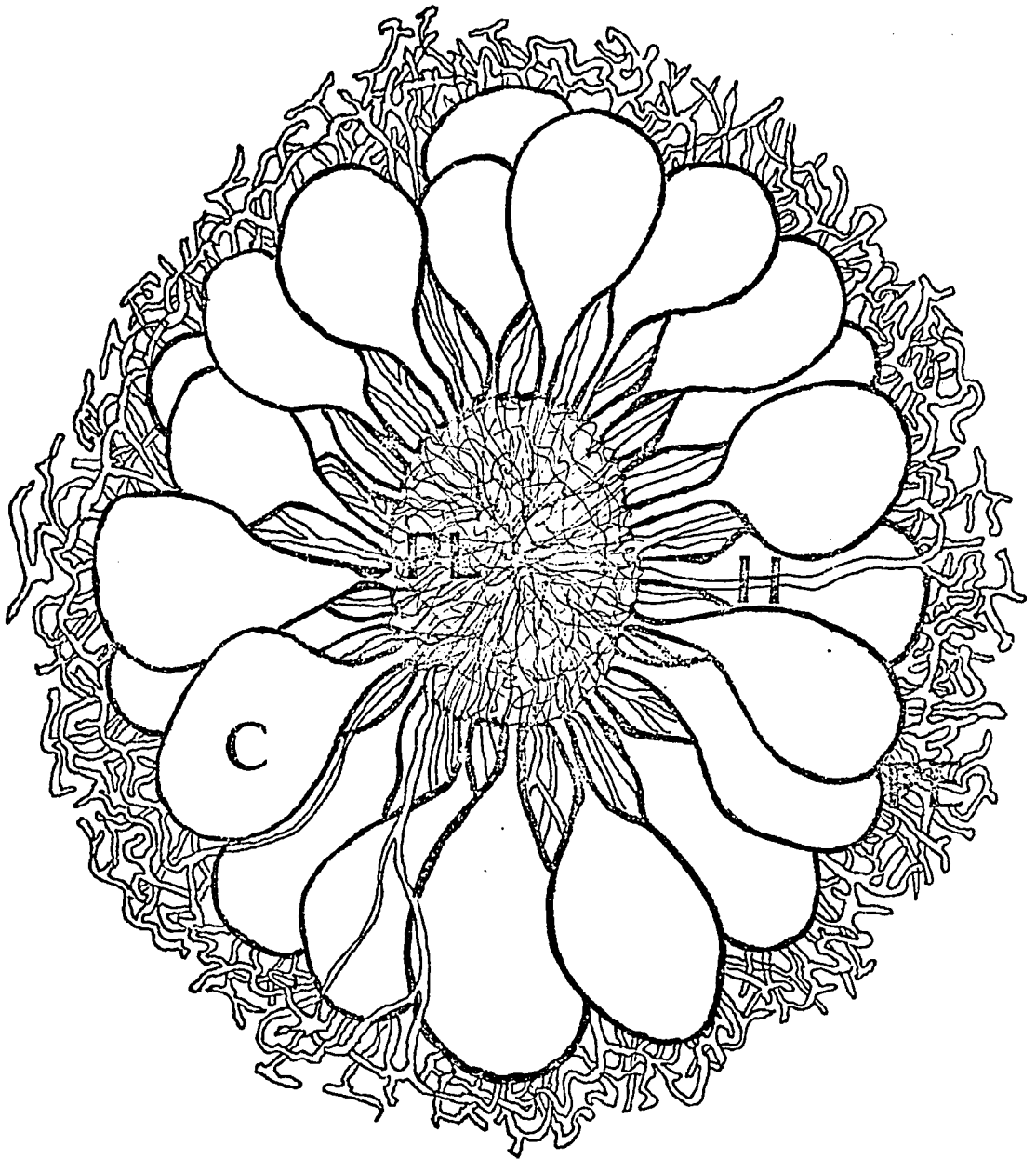
Glaziella Berkeley (1879/1880 as cited in Gerdemann & Trappe (1974)) This genus contains a single species, known only from the tropics. The spores are similar to those of Glomus, but are produced in the walls of sporocarps. The sporocarps are large, being up to 5 cm broad. The mycorrhizal status of Glaziella is unknown (Gerdemann & Trappe, 1974, 1975).

Sclerocystis Berkely & Broome (1875) This genus has chlamydospores characteristically arranged around a central plexus of sterile hyphae (Figure 13). Individual spores are indistinguishable from those of Glomus, and could be difficult to interpret if they became detached in extraction procedures.

There are six described species in the genus, two of which, S. coccogena (Pat.) von Hohn. and S. clavispora Trappe are unknown in the United States. One other species, S. dussii (Pat.) von Hohn. probably is tropical, but is known from greenhouses in the temperate zone (Gerdemann & Trappe, 1974). S. coremoides Berkeley



Figure 13. Diagrammatic representation of the Sclerocystis sporocarp. Note the sterile central plexus (PL), the radiating chlamydospores (C), and the peridium (PE). The peridium develops from hyphae that radiate from the plexus (H)



& Broome, S. rubiformis Gerdemann & Trappe, and S. sinuosa Gerdemann & Bakshi have all been found in the United States, and will be described in detail.

S. coremoides Berkeley & Broome (1875) is known from greenhouses in Oregon, and from field sites in Florida (Gerdemann & Trappe, 1974; Nicolson & Schenck, 1979). Sporocarps are subglobose to pulvinate, 340-600  $\mu\text{m}$  broad, and possess a peridium, 20-70  $\mu\text{m}$  thick, of interwoven hyphae, which is white to dull brown. Chlamydospores are obovoid-ellipsoid to oblong-ellipsoid and 50-86(-102) x 35-52(-82)  $\mu\text{m}$  in size. The sporocarps form epigeously in mats containing large numbers of specimens up to four sporocarps deep. The spores are arranged in a hemisphere on the sterile sporocarp base.

S. rubiformis Gerdemann & Trappe (1974) has dark brown subglobose to ellipsoid sporocarps, 180 x 180 - 375 x 675  $\mu\text{m}$  in size. There is virtually no peridium, and the clusters of spores resemble minute blackberries, hence the specific epithet. With this species, it is sometimes difficult to see the sterile plexus, thus S. rubiformis can be confused with small sporocarps of Glomus microcarpus. The individual chlamydospores are dark brown, 37-125 x 29-86  $\mu\text{m}$  in size, obovoid to ellipsoid or subglobose. The spore walls are laminated and 8  $\mu\text{m}$  or less thick.

S. sinuosa Gerdemann & Bakshi (1976) is very similar to S. coremoides. This species has sporocarps 248-412  $\mu\text{m}$  in diameter that are globose to pulvinate, with variably-shaped chlamydospores

45-118 x 30-83  $\mu\text{m}$  in size, both of which characteristics are similar in the two species. S. sinuosa, however, has a peridium only 6-20  $\mu\text{m}$  thick, composed of thick-walled, sinuous hyphae. The peridial hyphae of S. coremoides are not sinuous, and the peridium is much thicker (20-70  $\mu\text{m}$ ).

I have found both S. sinuosa and S. rubiformis in my collections from Iowa. All Sclerocystis species are thought to be endomycorrhizal (Gerdemann & Trappe, 1974).

Complexipes Walker (1979) gen. ined. This monospecific chlamydosporic genus is erected, and provisionally placed in the Endogonaceae as part of the work for this dissertation, and is fully described in Part 3 herein.

Modicella Kanouse (1936) (See Gerdemann & Trappe, 1974) Modicella is the only sporangial genus in the family. One of the two described species, M. reniformis (Bres.) Gerdemann & Trappe, is known only from South America, and will be excluded from this discussion.

M. malleola (Harkness, 1899) Gerdemann & Trappe (1974) has sporocarps, 2-5 mm broad, globose to subglobose or pulvinate. No peridium is present, and the sporocarp consists of basal radiating hyphae upon which the sporangia are formed. Sporangia are globose to ellipsoid to irregular, 55-118 x 50-91 in size, thin-walled, and hyaline. Sporangiospores are 7-17  $\mu\text{m}$  long, and subglobose to

ellipsoid, taking on a subangular form because of mutual pressure within the non-collumellate sporangium.

I found one specimen that is probably a few sporangia from a M. malleola sporocarp at Rhodes, Iowa. Walker (1923) reported the species from Nebraska, obtaining a culture, but failing to induce production of fruiting bodies. The mycorrhizal status of the genus is unknown.

For all the preceding descriptions, I have paraphrased the published work, although I have occasionally added information based on my own observations.

#### Population dynamics and species diversity of endogonaceous spores

Prior to the pioneering work of Mosse and Gerdemann, few workers recognized spores of the Endogonaceae in soil (Mosse, 1953, 1956; Gerdemann, 1955). Population studies did not commence until descriptions of the common species became available (Gerdemann & Nicolson, 1963; Gilmore, 1968; Mosse & Bowen, 1968a; Nicolson & Gerdemann, 1968).

With one exception (Nicolson & Gerdemann, 1968), "pre-Linnean" systems of nomenclature, or letter and number codes, were used in these early papers on spore occurrence. The complete revision of the family by Gerdemann & Trappe (1974) provided excellent descriptions and Latin names for all species known at that time. Interpretation of much of the work done, even in recent times (e.g., Abbott & Robson, 1977; Saif, 1975) nevertheless is hampered

by an insistence on retaining descriptive or coded names, rather than using Latin binomials. For example, the term "yellow vacuolate spore" (Mosse & Bowen, 1968a) was applied to Gl. mosseae, and has been used frequently in the literature (e.g., Khan, 1974; Mason, 1964; Mosse & Bowen, 1968b). Redhead (1977), however, used the term to refer to two different organisms in the same study, neither of which were Gl. mosseae. Similarly, the terms "red-brown laminate", "white reticulate", "bulbous vacuolate", etc. (Mosse & Bowen, 1968a), can each be used for a number of different taxa. Such problems can arise also with letter or number codes. Failure to lodge collections in herbaria has made it impossible to provide identification to species as taxonomic knowledge increased.

The hypogeous nature of most endogonaceous spores presents problems in extraction and enumeration. There are several different methods used to remove spores from the soil. The most common method is a wet sieving and decanting, in which different size screen meshes are used to separate spores from soil after their suspension in water (Gerdemann & Nicolson, 1963). A system depending on flotation of spores and their adhesion to glass surfaces also was described (Sutton & Barron, 1972). Other methods have been described, such as shaking or stirring soil vigorously in water, and, after a period of settling, decanting the supernatant with suspended spores (Saif & Iffat, 1976; Powell, 1977a); or pouring soil through density-gradients of liquid gelatin to suspend spores while allowing denser material to fall (Mosse &

Jones, 1968). All these methods have been used in studies of populations of endogonaceous spores. Other extraction methods, such as use of elutriators (Dr. J. Ruehle, U.S. Forest Service, Athens, Georgia (1975); pers. comm.), centrifugation with sugar density gradients (Ohms, 1957), and a glycerol bubbling-flotation method (Furlan & Fortin, 1975) have been used in other studies. These methods are tedious and time-consuming, and for population studies in Iowa, I used a centrifugation-sugar flotation method slightly modified from Jenkins (1964). Once spores are extracted, they are counted in suspension in Petri plates or nematode-counting dishes, or on fine-mesh screens, or filter papers. Most workers have used binocular dissecting microscopes at magnifications of 20-25X for counting. Such low magnifications, and the use of minimum mesh sizes of no less than 100  $\mu\text{m}$ , may have caused small-spored species to be overlooked (Ames & Linderman, 1977). Nicolson & Johnston (1979) overcame this by using a sieve with 75  $\mu\text{m}$  mesh, and pipetting spore suspensions onto microscope slides for examination through a compound microscope. In studies reported in this dissertation, counts were made from suspensions in Petri plates scored into squares, and, for the Rhodes Farm, where small-spored species are common, from subsamples picked out, placed on slides, and scanned through a compound microscope.

Comparison of the various studies on diversity of species and population levels (Table 7) is difficult because of the many different methods of sampling, extraction, and spore counting. In

Table 7. Species, diversity, and populations of endogonaceous spores.  
A.= Acaulospora, C.= Complexipes, Gi.= Gigaspora, Gl.= Glomus,  
S.= Sclerocystis

Reference	Country of origin	No. of sites sampled	Type of site	Extraction method	Smallest sieve ( $\mu$ m)
Mason 1964	Scotland	3 plots	Cultivated field	Wet sieving	104
Mosse & Bowen 1968b	Australia	134	Forest. Native grass and shrubs. Sown pasture and crops.	Wet sieving	100
Mosse & Bowen 1968b	England	3 plots	Cultivated field plots	Wet sieving	100
Shuja et al. 1971	Pakistan	13 angio-sperm trees	Ornamental trees on a campus	Wet sieving	104 <sup>b</sup>
Thapar & Khan 1973	India	6 locations	23 planted and natural trees	Wet sieving	104 <sup>b</sup>
Daft & Nicolson 1974	Scotland	3 sites	Coal wastes	Wet sieving	150

<sup>a</sup>Unless otherwise stated, use of fresh moist soil is assumed.

<sup>b</sup>Not stated, but inferred from text.

<sup>c</sup> $\bar{X}$  values are unweighted averages of the means expressed in the publications



Spore numbers <sup>a</sup>	Number of species per site	Species recorded	Notes
20-800/1	3	<u>Gl. caledonius</u> <u>Gl. geosporus</u> (?) <u>Gl. mosseae</u>	Species deduced from codes of Gerdemann & Nicolson (1963). Spore numbers estimated from figures.
not given	0-3	<u>A. laevis</u> <u>C. moniliformis</u> <u>Gi. calospora</u> (?) <u>Gl. geosporus</u> (?) <u>Gl. macrocarpus</u> (?) <u>Gl. mosseae</u> others	Subject to considerable doubts as to spp. Deduced from Mosse & Bowen (1968a)
not given	3-5	<u>Gi. calospora</u> (?) <u>Gl. geosporus</u> (?) <u>Gl. mosseae</u> others	Doubts as immediately above
not given	1-4	<u>Gi. calospora</u> (?) <u>Gl. geosporus</u> <u>Gl. mosseae</u> others	Doubts as for Mosse & Bowen (1968b)
40-1850/kg $\bar{X} = 373^c$	1-6	<u>A. laevis</u> <u>Gi. calospora</u> <u>Gi. gigantea</u> <u>Gl. geosporus</u> <u>Gl. mosseae</u> others	
not given	1-2	<u>Gi. calospora</u> <u>Gl. fasciculatus</u>	<u>Gl. tenuis</u> also present

Table 7 continued

Reference	Country of origin	No. of sites sampled	Type of site	Extraction method	Smallest sieve ( $\mu\text{m}$ )
Khan 1974	Pakistan	5 locations	Plains. Salt ranges. Coasts. Deserts.	Wet sieving	104 <sup>b</sup>
Crush 1975	New Zealand	7 sites	Cultivated & natural. Fertilized & not.	Wet sieving	100
Koske 1975	Australia	3 zones at one site	Sand dunes	Flotation-adhesion	
Saif 1975	Pakistan	73 plant species	Around a university campus	Flotation-adhesion	
Saif & Iffat 1976	Pakistan	8 sites. 127 plant species.	Native(?) grass & forests	Agitation-settling-decanting	
Abbott & Robson 1977	Australia	3 sites	Pasture. Native vegetation. Crop.	Wet sieving	106
Ames & Linderman 1977	U.S.A.	5 fields in Oregon and California	Lily fields	Wet sieving	45

Spore numbers <sup>a</sup>	Number of species per site	Species recorded	Notes
0-1110/kg $\bar{X} = 220$	1-3	<u>A. laevis</u> <u>Gl. mosseae</u> others	Doubts as for Mosse & Bowen (1968b)
1000-2000/l	1 (2 at one site)	<u>A. laevis</u> <u>Gl. fasciculatus</u>	<u>Gl. tenuis</u> also present
0-11000/kg $\bar{X} = 663$	1-4	<u>A. scrobiculata</u> <u>Gi. calospora</u> <u>Gi. heterogama</u> <u>Gi. gigantea</u> <u>Gl. geosporus(?)</u>	<u>A. scrobiculata</u> and <u>Gl. geosporus</u> deduced from descriptions in text
0-1980/kg $\bar{X} = 405$	0-3	<u>Gigaspora</u> spp. <u>Gl. geosporus(?)</u> <u>Gl. mosseae</u>	Doubts as for Mosse & Bowen (1968b)
0-51580/kg $\bar{X} = 3080$	0-4 "3-4 in most sites"	<u>A. laevis</u> <u>Gi. calospora</u> <u>Gl. fasciculatus</u> <u>Gl. macrocarpus</u> <u>Gl. mosseae</u> others	
0-4100/kg air-dried soil $\bar{X} = 728$	0-4	<u>A. laevis</u> <u>Gi. calospora(?)</u> <u>Gl. mosseae</u> others	Lower numbers in virgin soils. Doubts as for Mosse & Bowen (1968b). <u>Gl. tenuis</u> also present.
not given	1-4	<u>A. elegans</u> <u>A. trappei</u> <u>Gl. fasciculatus</u> <u>Gl. monosporus</u> G. & T.	Number of species varied within a site depending on time.

Table 7 continued

Reference	Country of origin	No. of sites sampled	Type of site	Extraction method	Smallest sieve ( $\mu\text{m}$ )
Johnson 1977	New Zealand	1 site 21 woody species. 13 herbs. (82 samples).	Forest	Unclear. Possibly gelatin columns	100 <sup>b</sup>
Powell 1977a	New Zealand	37 sites	Forest. Recent and developed pasture	Agitation-settling-decanting	53
Redhead 1977	Nigeria	9 sites. 36 samples.	Forest. Savanna	Wet sieving	100
Saif 1977	Pakistan	2 sites	Cultivated field. Nursery.	Agitation-settling-decanting	
Hayman 1978	New Zealand	35 sites	Forest. Native grass & scrub. Tussock. Clover. Pasture.	Wet sieving	50

Spore numbers <sup>a</sup>	Number of species per site	Species recorded	Notes
2-17000/1 $\bar{X} = 9345$	1-4	<u>A. laevis</u> <u>Acaulospora</u> sp. <u>Gi. calospora</u> <u>Gl. fasciculatus</u> <u>Gl. mosseae</u> <u>S. rubiformis</u>	
400-62000/1	1-5	<u>A. laevis</u> <u>Gi. calospora</u> <u>Gl. fasciculatus</u> <u>Gl. macrocarpus</u> <u>Gl. mosseae</u> <u>S. sinuosa</u>	
20-17284/1 $\bar{X} = 2710$	not clear	<u>A. scrobiculata</u> <u>C. moniliformis</u> <u>Gigaspora</u> spp. <u>Gl. fasciculatus</u>	Species unclear. Descriptive terms of Mosse & Bowen (1968a) used, but may not be same species.
800-2600/kg	not clear	<u>Gl. mosseae</u> others	Spore numbers estimated from graphs in paper
0-15900/kg air dried soil	0-6 (mean 3)	<u>A. laevis</u> <u>A. scrobiculata</u> (?) <u>Gi. calospora</u> <u>Gl. fasciculatus</u> <u>Gl. geosporus</u> <u>Gl. mosseae</u> <u>S. rubiformis</u> others	

Table 7 continued

Reference	Country of origin	No. of sites sampled	Type of site	Extraction method	Smallest sieve ( $\mu\text{m}$ )
Molina et al. 1978	U.S.A. and Canada	3 sites in W. Canada & 24 sites in W. U.S.A.	Native <u>Festuca</u> grassland	Wet sieving	104
Sward et al. 1978	Australia	1 area. 3 sites.	Disturbed and un-disturbed heathland	Wet sieving	100
Nicolson & Johnston 1979	Scotland	1 area. 4 sites.	Sand dunes	Wet sieving	75

Spore numbers <sup>a</sup>	Number of species per site	Species recorded	Notes
no data	1-5 (2.7-5 for different sites: 2.3-3.5 for plants within sites)	<u>A. laevis</u> <u>A. scrobiculata</u> <u>Acaulospora</u> sp. <u>Gl. calospora</u> <u>Gl. fasciculatus</u> <u>Gl. geosporus</u> <u>Gl. macrocarpus</u> <u>Gl. microcarpus</u> <u>Gl. mosseae</u>	<u>Gl. tenuis</u> also present
0-2400/kg	not clear	<u>A. laevis</u> <u>Gl. gigantea</u> <u>Gl. gilmorei</u> <u>Gl. margarita</u> <u>Gl. mosseae</u> others	Spore numbers estimated from graphs in paper
not clear (60-100 sporocarps each with 2-112 spores per 1 dry sand	1	<u>Gl. fasciculatus</u>	Compound microscope used for counting 5 ml suspension samples

addition, some workers expressed spore numbers in terms of soil volume; some in terms of soil dry weight; and some in terms of weight of moist soil. However, low diversity is common, most sites having less than five species. The highest number of species recorded is six, many sites having only one or two.

Spore density is extremely variable, and can reach both very high and very low levels. No overall trend of lower numbers in native ecosystems compared with disturbed or managed sites, or vice versa, as suggested by some workers (Abbott & Robson, 1977; Mosse & Bowen, 1968b) is apparent. Few unfumigated soils would lack sufficient natural inoculum for mycorrhizal establishment if the fungi present are capable of forming mycorrhizae and have viable spores.

Few publications deal with the dynamics of endogonaceous spore populations. The earliest study examined populations under three different crops over three growing seasons (Mason, 1964). No replication of samples was taken in the first year, and two replications were made in the following two years. Results showed seasonal variations, with total spore populations varying from 20-800/l. These data were not treated statistically, but populations were generally lowest in spring, and highest in autumn, although there were exceptions. With barley, for example, the spore population declined throughout the first year, and was lowest in November. Because of lack of adequate replication or statistical analysis, separation of sampling error from real differences is impossible.



A study of crops in Canada (Sutton & Barron, 1972) revealed relatively low numbers of spores in summer, followed by an increase in autumn, and generally static or declining populations through winter. Four replications were used in this experiment, but the species of fungi present were not identified other than by size and color. The authors suggested that a direct relationship between spore populations and root senescence existed, a conclusion that seems logically sound. Low numbers of spores in the early part of the growing season are possibly related to consumption by soil organisms, and germination of overwintered propagules. Two studies in Pakistan also showed low spore numbers in spring, gradually increasing through summer, and reaching highest values in autumn and winter (Khan, 1974; Saif, 1977).

A positive correlation between soil moisture and populations of spores may be possible. Such a relationship was suggested in an Australian study (Sward et al., 1978), although the relationship seemed to occur only on disturbed sites. Although two replications were used, no details of variation or statistical analysis were presented, excepting a statement: "Large local variations ... did not occur." Over the two-year period of study, highest populations were found in winter and spring, although for three of the five sites it appeared that little variation existed throughout the year. Studies in Nigeria indicated conflicting results, some sites showing high spore numbers in wet periods, and other locations showing no differences or the opposite effect. It is unclear if

true replicates were used, even though the author reported that "... at least 12 samples were pooled to represent any one site." (Redhead, 1977).

Generally, there appears to be a seasonality of populations, possibly related to root senescence, or rainfall, or both. Other factors, such as activity of soil organisms, cultivation practices, and fertilization also may have effects on endogonaceous spore populations. Considerable scope for population studies based on field surveys and experiments exists.

PART 1.

MYCORRHIZAE AND ASSOCIATED FUNGAL SPORES  
IN SEVEN HYBRID POPLARS POTTED IN FIVE IOWAN SOILS

Mycorrhizae and associated fungal spores  
in seven hybrid poplars potted in five Iowan soils

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## INTRODUCTION

The Department of Forestry, Iowa State University, is one of several groups under the leadership of the North Central Forest Experiment Station of the United States Forest Service, evaluating poplar selections and hybrids for use in the intensive, short-rotation culture of trees for fiber production. To assess growth differences, seven clones of poplars were potted as rooted cuttings in five different unsterilized Iowan soils, and grown for one year in a greenhouse. Since little is known about the mycorrhizae of poplars in the United States, it was decided to investigate the mycorrhizal condition of the plants, and to assess the species of endomycorrhizal fungi present in the soils after this growth period.

The experiment was not originally intended as a study of mycorrhizae, and hence neither soil nutrient analysis nor preliminary investigation of mycorrhizal fungal spectrum were made.

## MATERIALS AND METHODS

Seven clones of hybrid poplars were used in the study. Five of these were Populus x euramericana (Dode) Guinier; one was a natural hybrid between P. alba L. and P. grandidentata Michx.; and the seventh was a cross between P. tristis Fisch. and P. balsamifera L. (Table 1.1). These clones were originally selected because they had shown promise for intensive culture systems.

Four of the soils were from central Iowa, the fifth being from a site in N.E. Iowa, some 240 km distant. Two of the five soils were from woodland sites where P. grandidentata Michx. was the predominant plant. One soil was from an oak-hickory-elm woodland where Populus spp. were absent. The remaining two soils were from corn-soybean crop fields. One of the crop fields had been subjected to normal applications of agricultural fertilizers and herbicides. The other field reportedly had never been treated with agricultural chemicals (Table 1.2). All soils were collected in late autumn.

The poplar cuttings were rooted in sterile Jiffy Pots® under mist and then planted in 9 inch (23 cm) black polyethylene pots filled with a mixture of sterilized coarse sand and unsterilized soil (1:1 v/v). The sand served to reduce compaction in the pots. The pots were placed in a greenhouse in a randomized block design with five replications. The trees were watered as needed.

The pots were inspected at least twice a week, and all weeds removed as soon as they were discovered. No fertilizers were applied

Table 1.1. Details of the poplar clones grown in five Iowan soils for a year to assess mycorrhizal relationships

Clone name	Clone number <sup>a</sup>	Parentage	Origin	Genus Section <sup>b</sup>
Tristis #1	5260	<u>Populus tristis</u> x <u>P. balsamifera</u>	Saskatchewan, Canada	Tacamahaca
Negrito de Granada	5321	<u>P. x euramericana</u>	Ontario, Canada	Algeiros
Canada Blanc	5323	<u>P. x euramericana</u>	Italy <sup>c</sup>	Algeiros
Eugenii	5326	<u>P. x euramericana</u>	France <sup>c</sup>	Algeiros
I45/51	5328	<u>P. x euramericana</u>	Italy <sup>c</sup>	Algeiros
Wisconsin 5	5377	<u>P. x euramericana</u>	Wisconsin <sup>d</sup> , U.S.A.	Algeiros
Crandon	5339	<u>P. alba</u> x <u>P. grandidentata</u>	Iowa, U.S.A.	Leuce x Tremulae

<sup>a</sup>Clone number designated by the North Central Forest Experiment Station, Forest Service, U.S. Department of Agriculture, Folwell Avenue, St. Paul, Minnesota 55108, U.S.A.

<sup>b</sup>After Peace (1952).

<sup>c</sup>Clonal material obtained from trees in Ontario, Canada.

<sup>d</sup>Clonal material obtained from a tree in Iowa, U.S.A.

Table 1.2. Summary of the five Iowan soils to grow seven clones of hybrid poplar for a year to assess mycorrhizal relationships

Code	Woodland or non-woodland	Fertilized?	Origin
NP	<u>Populus</u> woodland	No <sup>a</sup>	Yellow River State Forest, Allamakee County
HP	<u>Populus</u> woodland	No <sup>a</sup>	Holst State Forest, Boone County
CNP	Crop field	Yes	Nevada area, Story County
WNP	Mixed woodland. No poplars.	No <sup>a</sup>	Nevada area, Story County
OCNP	Crop field	No <sup>b</sup>	Nevada area, Story County

<sup>a</sup>Assumed from undisturbed nature of woodland.

<sup>b</sup>Reported (C. D. Hertz, Department of Forestry, Iowa State University (1975); pers. comm.).



during the experiment.

After a year the trees were destructively assessed for biomass. The roots were washed free of soil in large tubs of water. Samples of the roots were fixed in formalin-acetic acid-alcohol (FAA) for later investigation of mycorrhizal condition. The soil washings in the tub were stirred vigorously and, after a settling time of one minute, a sample of the suspended solids was removed with a sieve (106  $\mu\text{m}$ ; 140 mesh). A subsample of the solids was added to a Petri plate half-full of water, and viewed through a binocular dissecting microscope at 25-50X magnification. Illumination was by combined incident and transmitted light, which allowed easy location of endogonaceous spores (Mosse & Bowen, 1968). Spores were picked out with stork-billed microdissecting forceps, and mounted in lactophenol on microscope slides for identification.

Root samples were cleared in KOH and stained in trypan blue in a modification of a method derived by Bevege (1968) for processing the dark roots of members of the Araucariaceae. The roots were washed clear of debris and autoclaved in 1N KOH for 7 min from the moment the pressure valve was closed. They were rinsed in fresh 1N KOH, and bleached with 10% Chlorox<sup>®</sup> (0.25% sodium hypochlorite) until the stele was visible clearly through the cortex. This was followed by a wash in clear water for 1 min. After being acidified in 1% HCL for 1 min, the roots were autoclaved in 0.25% w/v trypan blue in lactophenol. For staining, the valve

was closed and as soon as the autoclave reached  $15 \text{ lb/in}^2$  ( $103\,421 \text{ N/m}^2$ ) the steam was shut off and the pressure allowed to return to normal. Finally, the roots were destained in two changes of clear lactophenol. It was necessary to transfer the roots to the second bath of lactophenol after 12 h to insure all stain was extracted from the cortical cells. Destaining was complete after a few hours in the second bath.

Mycorrhizal formation was assessed by observation of cleared roots through a dissecting microscope at 50-100X magnification. Where there were doubts, or where mycorrhizae could not be seen by this method, specimens were mounted on microscope slides and studied under a compound microscope at magnifications up to 1250X. The abundance of mycorrhizae was assessed by a subjective scale of none (0), sparse (1), moderate (2), and abundant (3). All types of mycorrhizae present were recorded in this manner. The approximate colonization levels for the abundance levels were 1 = less than 15%; 2 = 15-60%; and 3 = greater than 60%. No account was taken of the density of endomycorrhizal vesicles, which varied greatly.

## RESULTS

Eight species of fungi from four genera of the Endogonaceae were recorded:

Entrophospora infrequens (Hall) Ames & Schneider

Gigaspora gigantea (Nicol. & Gerd.) Gerdemann & Trappe

Glomus constrictus Trappe

Gl. etunicatus Becker & Gerdemann

Gl. fasciculatus (Thaxter sensu Gerdemann) Gerd. & Trappe

Gl. macrocarpus var. geosporus (Nicol. & Gerd.) Gerd.

Trappe

Gl. mosseae (Nicol & Gerd.) Gerd. & Trappe

Sclerocystis rubiformis Gerd. & Trappe.

Glomus constrictus and Gl. macrocarpus var. geosporus were not separated. Indeed, it was not until the latter part of 1977, more than a year after this survey was done, that the former was named and shown to be different from the latter (Trappe, 1977). Quantification was not attempted, because it was neither possible to determine the quantity of soil from which each sample came, nor to take a precise amount of the suspended matter from each sieving.

No treatments lacked spores. The spore data were transformed by arcsine transformation, and analyzed by analysis of variance to examine the differences among treatments. The data used in clonal analysis were the proportion of replicates of each clone in which a species was recorded. For analysis among soils, the data were

the proportion of replicates in which the species was found for each soil.

All except three species of fungi showed no significant differences (Tables 1.3 and 1.4). For Gl. fasciculatus there was a difference among clones; and for Gi. gigantea and Gl. mosseae there were differences among soils. These differences were located by the Hartley sequential variant of the Q method of Newman & Kuels (Snedecor & Cochran, 1967). Clone 5339 had significantly fewer samples containing Gl. fasciculatus ( $p \leq 0.01$ ) than did any of the other clones, which did not differ significantly from each other. Soil OCNP yielded a significantly greater number of samples in which Gi. gigantea was present ( $p \leq 0.01$ ), and Gl. mosseae spores were found less frequently in soil NP than in other soils ( $p \leq 0.01$ ).

Three types of mycorrhizae were present (Figure 1.1). Vesicular mycorrhizae were the most common, being found in all clones, though only sparsely in clone 5339. No arbuscules were noted. Two kinds of ectomycorrhizae were found: a black one which agrees with the description of that formed by Cenococcum geophilum Fr. [= C. graniforme (Sow.) Ferd. & Winge] (Mikola, 1948); and a thin-mantled, light colored type formed by a septate fungus of unknown affinity. These ectomycorrhizae were found on clones 5260 and 5339 only, being most abundant on the former. Sclerotia very similar to those formed by C. geophilum (Trappe, 1969) were abundant in all soils, outnumbering spores by a factor of 10 or more.

Table 1.3. Fungal species found in five Iowan soils in which poplar clones were grown for a year in a greenhouse. Figures are mean number of replications (out of five), taken over all soils, in which a species was recorded. See Table 1.1 for clonal information

Poplar clone	Fungal species						
	<u>Entrophospora infrequens</u> <u>Gl. macrocarpus</u> v. <u>geosporus</u>	<u>Glomus constrictus</u> &	<u>Glomus etunicatus</u>	<u>Glomus fasciculatus</u>	<u>Glomus mosseae</u>	<u>Gigaspora gigantea</u>	<u>Sclerocystis rubiformis</u>
5260	4.2	2.0	0.2	2.8	3.0	0	0.2
5331	2.8	2.0	0	2.6	3.4	1.2	0
5323	4.0	2.4	0.4	3.8	3.0	0.8	0.2
5326	3.4	2.4	0	3.0	2.8	0.2	0
5328	3.4	1.8	0.4	2.8	3.2	0.2	0
5377	3.8	2.2	0	3.4	2.8	0.4	0.2
5339	3.0	0.4	0	0.2**	3.0	0.4	0

\*\*Significantly fewer spores for Gl. fasciculatus/5339. Otherwise no significant differences ( $p \leq 0.01$ ).

Table 1.4. Fungal species found in five Iowan soils in which poplar clones were grown for a year in a greenhouse. Figures are mean number of replications (out of five), taken over all clones, in which a species was recorded. See Table 1.2 for information on soils

Soil code	Fungal species						
	<u>Entrophospora infrequens</u>	<u>Glomus constrictus &amp; Gl. macrocarpus v. geosporus</u>	<u>Glomus etunicatus</u>	<u>Glomus fasciculatus</u>	<u>Glomus mosseae</u>	<u>Gigaspora gigantea</u>	<u>Sclerocystis rubiformis</u>
NP	3.28	2.00	0.14	3.43	1.00**	0.14	0.14
HP	3.71	1.29	0	3.00	3.71	0	0.14
CNP	3.71	0.86	0	2.00	3.14	0.14	0.14
WNP	3.14	2.70	0.42	2.00	2.71	0	0
OCNP	3.71	2.43	0	2.86	4.14	1.86**	0

\*\*Significantly lower ( $p \leq 0.01$ ) for NP/Gl. mosseae than for others in same column. Significantly higher ( $p \leq 0.01$ ) for OCNP/Gi. gigantea than for others in same column. Others within columns not significantly different.

Figure 1.1. Mean colonization levels and types of mycorrhizae found in seven poplar clones potted in five Iowan soils in a greenhouse for one year. Minimum = 0, maximum = 3. Endo = vesicular endomycorrhizae; Ecto 1 = black ectomycorrhizae, probably formed by Cenococcum geophilum; Ecto 2 = thin-mantled ectomycorrhizae, caused by a fungus of unknown affinity; Ectendo = ectendomycorrhizae. The four-digit numbers are clone numbers (see Table 1.1). Letter codes refer to soils (see Table 1.2)

## 5321 Endo

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NP	1.2
HP	2.0
CNP	3.0
WNP	1.4
OCNP	1.6

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## 5323 Endo

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NP	1.8
HP	1.8
CNP	3.0
WNP	1.6
OCNP	2.0

---

## 5326 Endo

---

NP	1.4
HP	2.0
CNP	2.6
WNP	1.0
OCNP	2.0

---

## 5328 Endo

---

NP	2.2
HP	1.6
CNP	3.0
WNP	1.6
OCNP	1.4

---

## 5377 Endo

---

NP	1.4
HP	1.6
CNP	3.0
WNP	1.2
OCNP	1.8

---

## 5260 Endo

---

NP	2.0
HP	2.6
CNP	2.8
WNP	1.4
OCNP	1.8

---

## 5339 Endo

---

NP	0.4
HP	0.2
CNP	0
WNP	0
OCNP	0.6

---

## 5339 Ecto

---

	1	2
NP	1.2	0
HP	1.2	0
CNP	0.6	0
WNP	0.6	1.2
OCNP	0.2	1.2

---

## 5260 Ecto

---

	1	2
NP	0	0
HP	0.4	0.4
CNP	0	0
WNP	0	0
OCNP	0	0

---

## 5339 Ectendo

---

NP	0
HP	0.6
CNP	0.4
WNP	0.6
OCNP	0.6

---

## 5260 Ectendo

---

NP	0.2
HP	0
CNP	1.0
WNP	0
OCNP	0.6

---



The third was an ectendomycorrhiza which occurred in a few of the plants of clones 5339 and 5260. It was found on long roots and bears a striking resemblance to the form described by Wilcox (1971). The hyphae and mantle of this ectendomycorrhiza were indistinguishable from those of the thin-mantled type of ectomycorrhiza found on the same two clones. Ectendomycorrhizae and ectomycorrhizae did not, however, occur on the same plant. Similarly, the two different ectomycorrhizae were not found on the same plant.

Similar statistical tests to those done on the spore data were performed on the mycorrhizal abundance figures. All the P. x euramericana clones were devoid of ectomycorrhizae and ectendomycorrhizae, and so no tests were needed for them. Clone 5339 had significantly lower scores for endomycorrhizae ( $p \leq 0.01$ ) than did all the other clones, which did not differ significantly (Table 1.5). Similarly, soil CNP had significantly more endomycorrhizae ( $p \leq 0.01$ ) than did the others (Table 1.6). For the black ectomycorrhizae, clone 5339 again had significantly higher scores than clone 5260 ( $p \leq 0.05$ ). There were no significant differences between clone 5339 and clone 5260 when figures for the thin-mantled ectomycorrhiza or the ectendomycorrhiza were compared.

Table 1.5. Overall mean mycorrhizal colonization levels for seven poplar clones grown for a year in a greenhouse in five Iowan soils. Minimum = 0, maximum = 3. For information about soils see Table 1.2. Endo = endomycorrhiza, Ecto = ectomycorrhiza, Ecten = ectendomycorrhiza

Clone <sup>a</sup>	Parentage of clone	Genus section <sup>b</sup>	mean colonization levels			
			Endo	Black Ecto	Thin-mantled Ecto	Ecten
5260	<u>P. tristis</u> x <u>P. balsamifera</u>	Tacamahaca	2.12	0.08	0.08	0.36
5331	<u>P. x euramericana</u>	Aigeiros	1.84	0	0	0
5323	<u>P. x euramericana</u>	Aigeiros	2.04	0	0	0
5326	<u>P. x euramericana</u>	Aigeiros	1.80	0	0	0
5328	<u>P. x euramericana</u>	Aigeiros	1.96	0	0	0
5377	<u>P. x euramericana</u>	Aigeiros	1.80	0	0	0
5339	<u>P. alba</u> x <u>P. grandidentata</u>	Leuce x Tremulae	0.24**	0.76*	0.48	0.44

<sup>a</sup>For information about clones see Table 1.1.

<sup>b</sup>After Peace (1952).

\*\*Significantly lower than others in same column ( $p \leq 0.01$ ).

\*Clone 5339 had more black ectomycorrhizae than clone 5260 ( $p \leq 0.05$ ).

Table 1.6. Overall mean mycorrhizal colonization levels by soils for seven poplar clones grown for a year in a greenhouse in five Iowan soils. Minimum = 0, maximum = 3. For information about clones and soils see Tables 1.1 and 1.2. Endo = endomycorrhiza, Ecto = ectomycorrhiza, Ecten = ectendomycorrhiza.

Soil	mean colonization levels			
	Endo <sup>a</sup>	Black <sup>b</sup> Ecto	Thin- <sup>b</sup> mantled Ecto	Ecten <sup>b</sup>
NP	1.48	0.60	0	0.10
HP	1.69	0.80	0.20	0.30
CNP	2.49**	0.30	0	0.70
WNP	1.17	0.30	0.60	0.30
OCNP	1.60	0.10	0.60	0.60

<sup>a</sup>All seven clones included in calculations.

<sup>b</sup>Clones 5260 and 5339 only included in calculations.

\*\*Significantly higher than others in same column. (Soil CNP was from a fertilized crop field).

## DISCUSSION

Mycorrhizae

Trappe (1962) listed 31 fungi as reportedly ectomycorrhizal with Populus species. Ten of these were referred only to the genus Populus. The remaining fungi were identified from 10 poplars; these being P. alba, P. angustifolia James, P. balsamifera, P. x canadensis Moench [= P. x euramericana], P. deltoides Marshall, P. nigra L., P. x robusta Schn., P. tremula L., P. tremuloides, and P. trichocarpa Torr. & Gray. Thus, the four major European and North American poplar sections (Leuce, Tremulae, Tacamahaca, and Aigeiros (Peace, 1952)) are shown to be capable of ectomycorrhizal development. The only fungus to occur as a mycotrophic partner with all these poplars is C. geophilum. Most of the 44 papers cited by Trappe as mentioning ectomycorrhizae of poplars were from the European literature, indeed, only 6 were from the American continents, and two of those referred to South America.

In this study, only two ectomycorrhizal types were recorded. There is no clue to the identity of the fungal partner in the thin-mantled type. It is quite possible that the fungus had airborne spores, and infested the soil after collection. Only growing plants in the soils under filtered air would clarify this. There were no signs of fruiting bodies in the pots.

The black mycorrhiza almost certainly was formed by C. geophilum,

a common and widespread symbiont (Trappe, 1964) that does not have airborne propagules. It is surprising that it did not form mycorrhizae with any of the P. x euramericana clones, because Dominik (1958) found it to be a frequent partner with P. x serotina Hartig [= P. x euramericana ], and it occurred in all of the taxa listed by Trappe (1962). If the sclerotia found were indeed those of C. geophilum, their abundance would indicate an adequate source of infection.

The data show differences between ectomycorrhizal establishment for different sections of the genus, although more representative groups would be needed to establish any consistency. Clone 5339 is a Leuce x Tremulae hybrid, and this was more readily ectomycorrhizal, at least with C. geophilum, than clone 5260 which is in the section Tacamahaca. Although the thin-mantled type did not show equivalent differences, the data show a suggestion of a similar trend for this mycorrhiza. The lack of significance may be because of the low numbers involved. Under the circumstances prevailing, the Aigeiros representatives did not form ectomycorrhizae. There is no hint of why this is so, unless there is possibly an inverse relationship with endomycorrhizal colonization.

As with the ectomycorrhizae, the Aigeiros poplars lacked ectendomycorrhizal colonization, and where it did occur, on the two remaining clones, it was infrequent. It did occur in all soils, but as with the thin-mantled ectomycorrhiza, it may have been a

result of airborne inoculum. In pines, ectendomycorrhizae are found normally only in stressful conditions where, perhaps, their causal fungus can compete better than other mycorrhizal fungi (Mikola, 1965). Ectendotrophic mycorrhizae occurred on P. x euroamericana on coal spoils in Czechoslovakia (Mejstrik, 1971), and a mycorrhiza similar to the ectendomycorrhizae reported here occurred on poplars from mineral mine tailings in Michigan, though it was considered to be an ectomycorrhiza (Harris & Jurgensen, 1977). Both these reports are from unnatural conditions, and the equally unnatural conditions prevailing in this study may have been instrumental in allowing ectendomycorrhizae to form.

Among the European workers, Melin (1923) described a mycorrhiza from P. tremula which is clearly a compound organ made up of an ectomycorrhiza and an endomycorrhiza in the same root. He was not, however, the first to show that poplars could be endomycorrhizal. Indeed, one of the earliest detailed reports of a phycomycetous endomycorrhiza was from a poplar, probably P. nigra var. italica Du Roi (Dangeard, 1900). European studies have shown that endomycorrhizae form in P. alba, P. nigra, P. alba x P. nigra, P. nigra x P. alba, P. x euramericana, P. x berolinensis Dippel, and P. x marilandica Bosc. (Dominik, 1958; Fontana, 1961; Mejstrik, 1971). In North America, endomycorrhizae have been observed on P. deltoides Bartr. (Lohman, 1927; Vozzo, 1969; Vozzo & Hacskeylo, 1974) and P. tremuloides (McDougall & Jacobs, 1927).

All taxa tested in this study formed vesicular mycorrhizae. No arbuscles were found, which is surprising because Gigaspora gigantea spores were found in the soils, and this species causes arbuscular mycorrhizae devoid of vesicles (Nicolson & Gerdemann, 1968). Clone 5339 formed endomycorrhizae only rarely, and then had sparse infection. There were no significant differences among the other clones. Thus, the Aigeiros and Tacamahaca group did not differ, while the Leuce x Tremulae hybrid showed a reluctance to combine with members of the Endogonaceae. The latter is in agreement with the findings of Dominik (1958), who sampled aspen x white poplar hybrids at eight different sites without finding endomycorrhizal colonization.

The only soil in which significant differences in mycorrhizal infection occurred was CNP, endomycorrhizal infection being significantly higher than for other soils ( $p \leq 0.01$ ). This soil is the only one with known fertilizer application. This result is somewhat at variance with previously reported reactions to fertilizers, where mycorrhizal infection decreased with increasing fertility levels (Daft & Nicolson, 1966, 1969). However, the mineral nutrient levels in the soils were not measured, and it is speculation that nutrient levels for soil CNP were higher than for the other soils.

#### Fungal species

Of the 9 fungi identified, one, C. geophilum, is a common ectomycorrhizal fungus. Vozzo & Hacskeylo (1974) reported mycorrhizae of

P. deltoides caused by C. geophilum which correspond exactly to those found in this study. It is surprising, therefore, that such a symbiosis was not formed with the P. x euramericana clones, since one of their parents is P. deltoides. C. geophilum has been recorded as a symbiont with P. x euramericana on two occasions (Dominik, 1958 and Mejstrik, 1971).

The remaining 8 fungi are all members of the Endogonaceae (Zygomycetes: Mucorales). There is apparently only one published account of a known species in the Endogonaceae associated with poplar endomycorrhizae (Glomus macrocarpus Tul. & Tul. var. macrocarpus Gerd. & Trappe) (Dominik & Ihnatowicz, 1979). Vozzo & Hacskeylo illustrate "Endogone" spores, but it is impossible to identify them beyond genus. Their "translucent yellow-brown spore with halves separated by fissure" does not agree with any published description of a member of the family, and possibly is an undescribed Glomus sp. Their "aggregates of spores" may be a Sclerocystis sp., but the illustration is inadequate to confirm this.

Although it was not possible to quantify accurately the spore populations, one clone, 5339, yielded extremely low spore numbers. Whereas, with the other clones, a gram or two of suspended solids contained 50-100 spores, the same quantity from clone 5339 normally yielded less than 10. This is not surprising considering the extremely low incidence of endomycorrhizae in that clone (Figure 1.1). Had a quantitative experiment been done, it is likely that there would



have been more significant differences between clone 5339 and the other clones than was shown by the presence or absence data collected.

All but one of the endogonaceous species found in this study form endomycorrhizae with flowering plants. The exception is Entrophospora infrequens. We have been unsuccessful in attempts to establish E. infrequens in pot culture, and its mycorrhizal associations remain unknown (Hall, 1977; Ames & Schneider, 1979).

In relation to the soils, there were two significant differences in spore populations. Gigaspora gigantea occurred with a greater frequency in the crop soil without chemical usage (OCNP) than in the others. It was absent from soils CNP and WNP, but the low frequency in the other soils indicates that this may have been due to inadequacies of sampling technique, rather than an absolute absence from the soils. Glomus mosseae occurred less frequently in soil NP. This soil was from a different area of Iowa from the others, and perhaps this difference reflects intraspecific differences in sporulation.

Taking the overall occurrence of spores for each soil, and testing them with Kendall's Coefficient of Concordance and  $\chi^2$  (Siegel, 1956), the ranking of species was not significantly different ( $p \leq 0.01$ ). It was, in descending order of abundance, E. infrequens, Gl. mosseae, Gl. fasciculatus, Gl. macrocarpus var. geosporus and Gl. constrictus, Gi. gigantea, Gl. etunicatus, and S. rubiformis. The same test on the ranking of fungal species by clones produced exactly the same overall result.

## CONCLUSIONS

This study confirms that all three major types of mycorrhiza (ecto-, endo-, and ectendo-) occur in the genus Populus, and associates identified species in the Endogonaceae with poplar endomycorrhizae.

The results suggest that there may be differences in mycotrophic behaviour between different sections of the genus Populus. The representative of the Tacamahaca group (clone 5260) appears to be indiscriminating, and readily forms all types of mycorrhizae. The Leuce x Tremulae hybrid (clone 5339) was singularly reluctant to form endomycorrhizae, but formed ecto- and ectendomycorrhizae. The remainder of the clones were P. x euramericana, from the section Aigeiros, and these formed abundant endomycorrhizae, but failed to form either of the other types. There were no differences among clones in this taxon.

Clone 5339 showed an interesting reluctance to form endomycorrhizae with Glomus fasciculatus which sporulated sparsely with this hybrid, and presumably, therefore, had not formed mycorrhizae very successfully. This fungus is usually considered to be non-host-specific, and is one of the most common and widespread endomycorrhizal symbionts.

Differences in fungal spectrum among the five soils were not marked. Only two endogonaceous species showed population differences, and these were for only one soil in each instance. Some of the soils failed to yield specimens of some species (Table 1.4), but

this may have been because of inadequacies of the sampling technique. Future experiments of this type should include careful re-sampling of such soils, as well as pre-sampling for species present at the outset. The opportunistic nature of this survey (see introduction) precluded such efforts in this instance.

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## PART 2.

POPULATIONS OF ENDOGONACEOUS FUNGI  
IN TWO LOCATIONS IN CENTRAL IOWA



Populations of Endogonaceous fungi  
in two locations in central Iowa

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## INTRODUCTION

As part of cooperative research by Iowa State University and the North Central Forest Experiment Station, U.S.D.A. Forest Service on intensive culture of hybrid poplars for fiber production, four clones of poplar hybrids, planted at two locations in central Iowa, were investigated for mycorrhizal status and development (H. S. McNabb Jr. & C. Walker, in preparation). At the same time, samples of rhizosphere soil were taken and populations of endogonaceous spores examined. At one site, unplanted land, with and without plowing and herbicide treatment, also was investigated for populations of spores of endomycorrhizal fungi. Poplars are known to form endomycorrhizae as well as ectomycorrhizae and ectendomycorrhizae (Dominik, 1958; Levisohn, 1957; Lohman, 1927; Mejstrik, 1971; Vozzo & Hacskeylo, 1974). Endomycorrhizae may be especially important to poplars in their earliest years of establishment (Dominik, 1958).

Many endomycorrhizal fungi in the Endogonaceae produce abundant resting spores in soil (Gerdemann & Trappe, 1974), and these may be important as propagules for initiating endotrophic symbioses (Gerdemann, 1955; Mosse, 1956, 1972). Knowledge of the species, diversity, distribution, and population levels of these spores may be useful when soil treatments and planting strategies are considered. Application of herbicides is a common pre-planting treatment. Effects of such treatment on mycorrhizal fungi are unknown, but may be important if the treatment results in population changes. There are indications that conversion from native grassland to pasture,

presumably by plowing, leads to depression in spore production and change in species diversity (Hayman, 1978).

## MATERIALS AND METHODS

Hybrid poplars that had shown promise for intensive culture systems (U.S. Forest Service, 1976) were studied. One is a natural cross between Populus alba L. and P. grandidentata Michx. ("Crandon": NC #5339<sup>1</sup>). One, "Tristis #1": NC #5260, is a hybrid between P. tristis Fisch. and P. balsamifera L. The remaining two ("Canada Blanc": NC #5323 and "Eugenii": NC #5326) are black poplar hybrids (P. x euroamericana (Dode) Guinier). Preliminary investigations had shown qualitative and quantitative differences in mycorrhizal associations among these clones (C. Walker & H. S. McNabb, Jr., in preparation).

The two locations used for study have differing characteristics. The first (used in 1977), at the 4H Camping Center, Boone County, is a sandy terrace near the Des Moines River, well above flood level and not, therefore, subject to inundation. The soil has little structure, A and B horizons not readily being evident. A poorly growing crop of white ash (Fraxinus americana L.) occupied the land, most of the trees being less than 3 m high. Poor survival had resulted in uneven spacing and the trees had not closed canopy. There was a dense cover of mixed herbaceous plants as well as scattered seedlings of oak (Quercus sp.), elm (Ulmus sp.) and red cedar (Juniperus virginiana L.).

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<sup>1</sup>NC # is the clone number allocated by the North Central Forest Experiment Station, Forest Service, U.S. Department of Agriculture, Folwell Avenue, St. Paul, Minnesota 55108 to poplars in the intensive culture program.

The second location, an old meadow near Rhodes, Marshall County (studied in 1978), is situated approximately 56 km due east of the 4H site. The soil is a Nodaway silt-loam with C horizon at a depth of more than a meter. The plant community prior to treatment consisted of well-established mixture of smooth brome (Bromus inermis Leyss.) and foxtail grasses (Setaria spp.). The land is on the flood plain of a small stream and is subject to intermittent inundation, especially in spring and early summer.

A dense herbaceous cover is implicative of an abundant source of endomycorrhizal inoculum, and preliminary examinations revealed large and varied populations of endogonaceous spores. Similarly, in mature oak woodland near both sites, seasonally abundant sporocarp populations could be expected as sources of ectomycorrhizal inoculum. Sporocarps of the Tuberaceae were common in soil around the oaks. Mycorrhizae have been synthesized between P. x euroamericana and Tuber albidum Pico (Fontana & Palenzona, 1969), and one of us (Walker) has found Tuber murinum Hesse sporulating abundantly around ectomycorrhizae of clone NC #5323 in central Iowa, one sporocarp being traced to the mantle through hyphal connexion.

Cuttings of poplars were rooted in Jiffy Pots® under mist and hardened by gradual reduction of the misting regime. Because Crandon cuttings root poorly, they were dipped for 5 sec in 500 ppm aqueous solution of IBA. The other clones did not require such treatment. Root samples examined prior to planting showed the plants to be non-mycorrhizal. Plants in their Jiffy Pots® were transplanted to research

plots, with the tops of the pots about 2 cm below the soil surface.

The ash trees on the 4H site were removed and a rotary cultivator used to incorporate the vegetation and prepare a fine tilth to a depth of about 30 cm. Plots were staked and a soil sample taken from each to assess initial spore populations. Eight blocks, each containing a plot of each clone were planted. In each plot the plants were spaced at intervals of 1 m in a 5 x 5 matrix. For insurance against losses in early establishment, five extra trees were planted near those trees that had been pre-selected randomly for the earliest samplings. Thus, each plot consisted of 30 trees of a single clone. An unplanted buffer row of 4-m width was left between plots. Prior to planting plots were sprayed with 5.6 kg/ha of Lorox® to suppress weed regrowth. The blocking was done to take account of shading by oak trees on the western edge of the site.

The Rhodes site was marked into 32 plots, each 14-m square. Immediately prior to ground preparation, two random soil samples were taken from each plot, combined and examined for spore populations. Four replications of each treatment were randomly located within the site without blocking. The treatments were (1) untreated land (U); (2) unplanted plowed land (P); (3) unplanted land treated with herbicide (H); (4) unplanted land with both plowing and herbicide treatment (U + H); (5) P + H with Tristis #1; (6) P + H with Canada Blanc; (7) P + H with Eugonii; and (8) P + H with Crandon. The trees were planted in a 6 x 6 matrix at 2-m spacing. No untreated strips were left between plots, but samples were taken only from the inner 16 trees or from the central 6 m x 6 m area of the

unplanted plots. Ground preparation was by mold-board plow and disc-harrow, because the soil was too heavy for the rotary cultivator. The herbicide treatment consisted of a mixture of Lorox® at 5.6 kg/ha and Roundup® at 2.34 l/ha.

At planting, a random soil sample was taken from each plot. A core approximately 10 cm diameter and 18 cm deep was removed with a small trowel, placed in a plastic bag, and stored at 2°C until it could be processed. Subsequent samples from the unplanted plots at Rhodes were taken in a similar manner. Throughout the sampling, great care was taken to avoid cross-contamination of samples. All tools were washed in clean water after each sample was taken.

Two weeks after planting, and every two weeks thereafter until fall, a tree was randomly sampled, without replacement, in each planted plot. Root and soil samples were taken; the former for analysis of the progress of mycorrhizal colonization, and the latter for the spore survey. The surface soil was scraped along with the roots of any weeds, some of which had grown despite herbicide treatment. About 500 g of soil was taken from the rhizosphere of the tree. The soil was a combination of three subsamples, the first from around the base of the tree, the second from half way along the root being sampled, and the third from the tip of the root. On the first two sampling dates after planting, the roots had not grown sufficiently, so the sample was taken from directly beneath the tree.

Spores were extracted from soil by use of a centrifugation

technique modified from that used for extracting nematodes from soil (Jenkins, 1964). The sample was mixed thoroughly and all peds broken. Two subsamples, each about 110 ml in size, were taken and weighed. One subsample was used for calculating gravimetric moisture content and pH; the other, for extraction of spores. Soil for spore extraction was added to a bucket half-full of water and mixed thoroughly. All small lumps of soil were carefully broken and the resultant suspension stirred vigorously, allowed to settle for 15 s, and then gently decanted through a 24-mesh screen (710  $\mu\text{m}$ ) into another bucket. The debris on the sieve was scanned for the presence of sporocarps and discarded. No sporocarps were found. The suspension in the second bucket was swirled vigorously and allowed to settle for 15 s. The supernatant was gently poured through a 325-mesh sieve (45  $\mu\text{m}$ ) and the material on the sieve backwashed into a beaker. The suspension in the beaker (usually about 25 ml of soil and 55 ml of water) was stirred and transferred to two 50-ml plastic centrifuge tubes. The tubes were balanced with water and spun for 3 min at 895 G on a horizontal centrifuge. Attempts to repeat the procedure with a fixed-head, angled centrifuge resulted in poor retrieval of spores. The supernatant was discarded and the tubes filled with sugar solution and stirred thoroughly. To make the sugar solution, sucrose (household sugar) was added to 700 ml water until a liter of solution was obtained. The tubes were then balanced with sucrose solution and centrifuged for 15 sec at 895 G. The supernatant was poured onto a 400-mesh sieve (38  $\mu\text{m}$ ) and sluiced with water to remove the sugar.



Spores, nematodes, sclerotia, and other such material retained on the sieve could be washed into dishes for examination, or jars for storage. Extracted spores were stored at 2°C in Ringer's solution (Daniels & Graham, 1976) until counts could be made for the 1977 survey. The Rhodes soil, however, had high fungal activity, and spore counts were impossible because of rapid growth of contaminants. The data for the time of planting were unfortunately lost because of this contamination. Later samples were stored in 5% formalin, which satisfactorily overcame the problem.

For the 4H survey, spores were washed into a plastic Petri plate (8.5 cm diameter) which had been scored into squares to fit the field of view of a dissecting microscope at a magnification of 20X. The plate was systematically scanned and all spores counted. A mixture of incident and transmitted light was used for illumination (Mosse & Bowen, 1968a). The scheme of morphological classification of these authors was used to place the spores into classes. Some of these classes later proved to be either a mixture of taxa, or a developmental stage of a taxon. However, the spores could be identified to their class with accuracy by this method. Representative examples of spores were preserved in lactophenol mounts for future reference, and later identified to species.

Many of the spores from the Rhodes site were less than 100  $\mu\text{m}$  in diameter. These spores were impossible to identify under a dissecting microscope. Consequently, a different method was devised to estimate populations. The spores were washed into a Petri plate

(5 cm diameter), that had been scored into 1 cm squares. The plate was systematically scanned under a dissecting microscope at 20X magnification, and the first 50 specimens too small to identify were deposited in a drop of lactophenol on a microscope slide. A cover slip (18 mm square) was placed on the slide and the spores counted and identified under a compound microscope at 125X. The slide was systematically scanned from top to bottom. The remaining small spores were counted, but not identified. The ratio of different small-spored species on the slide was used to calculate their abundance in the dish from the total count. Comparisons of this method with total counts made under the compound microscope showed no significant errors. Large spores were never abundant, so they were all picked onto another slide for counting and identification.

## RESULTS AND DISCUSSION

Recognition of species, based on the concepts of Gerdemann & Trappe (1974), was possible once an understanding of the taxonomy of the Endogonaceae had been acquired. The morphological characterization of spores at the 4H site clearly became inadequate. The "bulbous vacuolate" group consisted of spores of Gigaspora calospora (Nicolson & Gerdemann) Gerdemann & Trappe, Gi. gilmorei Trappe & Gerd., and mature spores of Gi. rosea Nicolson & Schenck. The "bulbous opaque" category consisted solely of young spores of Gi. rosea. From the fourth sampling date, two separate categories of "sessile" spores had been recognized, which later proved to be different age-groups of Acaulospora scrobiculata Trappe. At the first three counts these had not been separated, the older stage not being recognized as a member of the Endogonaceae. The initial collection had been kept, and was re-counted, but the second and third collections had been discarded, thus these data were lost. A similar problem arose for spores in the Glomus fasciculatus (Thaxter sensu Gerd.) Gerd, & Trappe group. These were rather small and light-colored, and were not recognized until the fourth sampling. Consequently, the figures for A. scrobiculata and Gl. fasciculatus for times 2 and 3 are linear interpolations between the first and fourth dates, and are therefore subject to doubt. By the time the Rhodes survey was started, an adequate knowledge of the taxonomy of the group allowed identification to species at time of counting.

Survey of the 4H site over two growing seasons had been intended, but deer destroyed a large proportion of the trees. Block 8 was damaged so badly that insufficient trees remained for completion of the study during the first season. Consequently, the survey was for a single season only, and block 8 was eliminated from statistical analysis. No such problems arose at the Rhodes site because the area was fenced against deer.

In a different modification of the centrifugation-flotation extraction technique (Jenkins, 1964), Smith & Skipper (1979) found that almost 94% of spores extracted from a small sample of soil were found in the water after the first centrifugation. With the technique used in our studies, spores were rarely found in the first supernatant, and when found, the spores usually contained gas bubbles and appeared dead. The difference is puzzling, but may be a result of the larger quantities of soil (110 ml vs 10 g) and the higher centrifugal force (895 G vs 412 G) used in our method. Preliminary trials showed our method gave good agreement between subsamples from the same thoroughly mixed soil sample. We do not know, however, what proportion of the total spore population was extracted, or if the proportion was the same for each species or soil type. Comparisons among species should be viewed with this in mind, and figures presented should be considered as minima, rather than as absolute values.

## Species diversity<sup>1</sup> and distribution

Ten species of the Endogonaceae were found at the 4H site and 12 at the Rhodes site, including two species of Acaulospora, two of Gigaspora, and three of Glomus common to both sites (Table 2.1). Two species of Gigaspora and one of Glomus found at 4H did not occur at Rhodes. Similarly, Rhodes yielded one Acaulospora species and four Glomus species that were not found at the other location. Among the 15 species found there were three undescribed taxa, one of which, A. spinosa Walker & Trappe sp. ined., was abundant at the 4H site, but so rare at Rhodes that records of its occurrence were not kept.

Within a site, the spatial distribution of spores was extremely uneven, and rarely were all species recorded from a single soil sample. At Rhodes, the range was 3-11 species per sample, with a mean of 7.45. All 11 species were found in only five of the 288 samples. Examined over all times, eight plots had 10 species, the remaining 24 yielding the full complement of 11 (Figure 2.1). At any one sampling date, the full complement of species was obtained.

The confusion over Gigaspora species in the 4H survey made it impossible to be precise about the number of species in each 4H sample. However, excluding times 2 and 3 and the group containing Gi. calospora and Gi. gilmorei, the mean number is 4.93 species (range 2-8) for any one sample. Over all times (Figure 2.2), the mean number of species found in a plot was 7.16 (range 6-8), and, as at Rhodes,

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<sup>1</sup>Simple species diversity, i.e., the numbers of species in a sample or site.

Table 2.1. Species of Endogonaceae found in surveys of soil collections made at two field sites in central Iowa. Voucher specimens have been deposited in the Iowa State University Herbarium (ISC)

Fungal species recorded at the 4H site	Fungal species recorded at the Rhodes site
<u>ACAULOSPORA</u> spp.	
<u>A. scrobiculata</u> Trappe	<u>A. scrobiculata</u>
<u>A. spinosa</u> Walker & Trappe sp. ined.	<u>A. spinosa</u>
	<u>A. trappei</u> Ames & Linderman
<u>GIGASPORA</u> spp.	
<u>Gi. calospora</u> (Nicol. & Gerd.) Gerd. & Trappe	<u>Gi. calospora</u>
<u>Gi. gilmorei</u> Trappe & Gerdemann	<u>Gi. gilmorei</u>
<u>Gi. rosea</u> Nicolson & Schenck	
<u>Gi. heterogama</u> (Nicol. & Gerd.) Gerd. & Trappe	
<u>GLOMUS</u> spp.	
	<u>Gl. albidus</u> Walker & Rhodes sp. ined.
<u>Gl. constrictus</u> Trappe	
	<u>Gl. epigaeus</u> Daniels & Trappe
<u>Gl. fasciculatus</u> (Thaxter sensu Gerd.) Gerd. & Trappe	<u>Gl. fasciculatus</u>
<u>Gl. geosporus</u> (Nicol. & Gerd.) Walker stat. ined. <sup>a</sup>	<u>Gl. geosporus</u> <sup>a</sup>
	<u>Gl. microcarpus</u> Tul. & Tul.
<u>Gl. mosseae</u> (Nicol. & Gerd.) Gerd. & Trappe	<u>Gl. mosseae</u>
	<u>Gl. occultus</u> Walker sp. ined.

<sup>a</sup>= Glomus macrocarpus var. geosporus (Nicolson & Gerdemann)  
Gerdemann & Trappe.

Figure 2.1. Distribution by space and time of endogonaceous spores in soil at Iowa State University Rhodes Farm, Rhodes, Marshall Co., Iowa. Each species of fungus is represented over space by a plan of the site with the sample plots represented by 32 squares. The height of the black bar within a square is proportional to the number of times the species was found in the plot over the sampling period (June to November, 1978). A. = Acaulospora; Gi. = Gigaspora; Gl. = Glomus

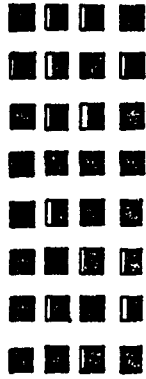
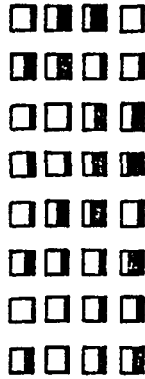
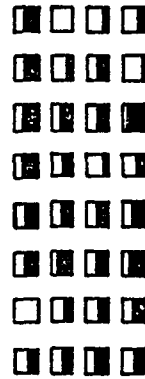
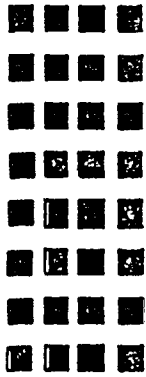
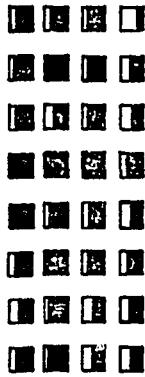
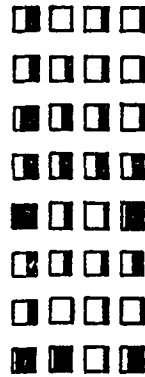
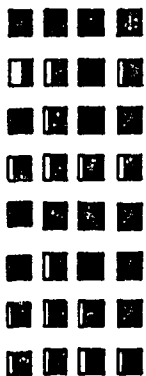
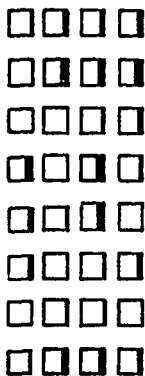
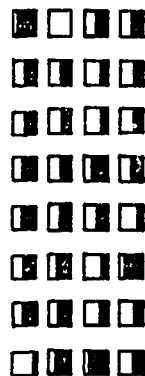
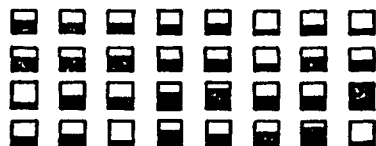
Gi. calosporaGi. epigaeusGi. microcarpusGi. occultusA. trappeiGi. albidusGi. geosporusA. scrobiculataGi. gilmoreiGi. fasciculatusGi. mosseae



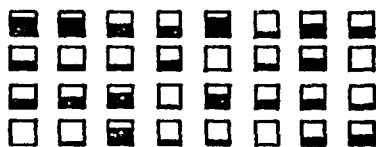
Figure 2.2. Distribution by space and time of endogonaceous spores in soil at the 4H Camping Center, Boone Co., Iowa. Each species of fungus is represented over space by a plan of the site with the sample plots represented by 32 squares. The height of the black bar within a square is proportional to the number of times the species was found in the plot over the sampling period (June to November, 1977).  
A. = Acaulospora; Gi. = Gigaspora; Gl. = Glomus



A. spinosa



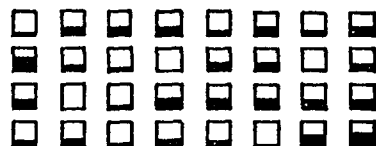
Gl. geosporus



Gl. heterogama



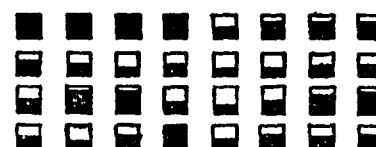
Gl. spp.



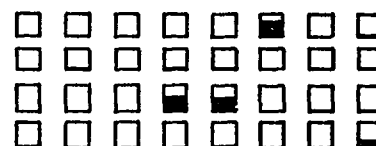
Gl. mosseae



Gl. fasciculatus



Gl. rosea (mature)



Gl. constrictus



A. scrobiculata

over the 32 plots at any one date, all species were found, although Gl. constrictus often was found in only one or two plots. The two excluded Gigaspora species were very common, and probably would have increased these numbers by almost two.

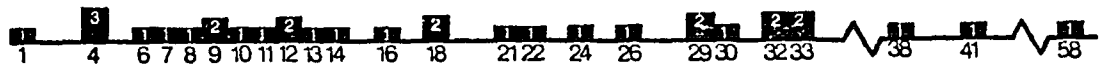
The species diversity at the two sites was higher than those given in previous reports. Between three and five species commonly have been reported (Abbott & Robson, 1977; Ames & Linderman, 1977; Johnson, 1977; Ksike, 1975; Molina et al., 1978; Mosse & Bowen, 1968b; Powell, 1977; Shuja et al., 1971). Several studies have reported only 1-3 species per site (Crush, 1975; Daft & Nicolson, 1974; Khan, 1974; Mason, 1964; Mosse & Bowen, 1968b; Nicolson & Johnstone, 1979; Saif, 1977). Only Hayman (1978) and Thapar & Khan (1973) reported more than five species at any one site. In the former, a study of 35 sites in New Zealand, the mean number was only 3, with a range of 0-6. In the latter, 1-6 species were found (mean 4.4) from soil supporting 23 tree species in six locations in India.

Numerically, the species of spores were distributed over the two sites in clumped distributions which rarely approached normality, and often were tending toward the negative binomial. Frequency distributions were plotted (Figure 2.3) for each species at each time. Usually, most species occurred in low numbers, but high and very high population clumps occurred.

The uneven distribution of spores over time and space implies that large numbers of sample replications are needed to ascertain

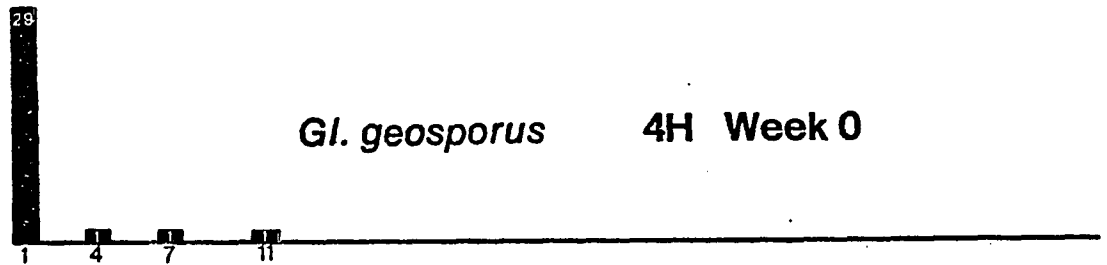
Figure 2.3. Representative examples of frequency distributions of endogonaceous spores in the soil at two sites in central Iowa (4H Camping Center, Boone Co. in 1977, and Iowa State University Rhodes Farm, Marshall Co. in 1978). Classes are hundreds of spores per kg of oven dry soil (black figures). Bar heights represent frequencies of classes out of 32 samples (white figures). A. = Acaulospora, Gl. = Glomus. Week is number of weeks after poplar rooted cuttings were planted in some of the plots in early July

F

*Gl. fasciculatus* 4H Week 14

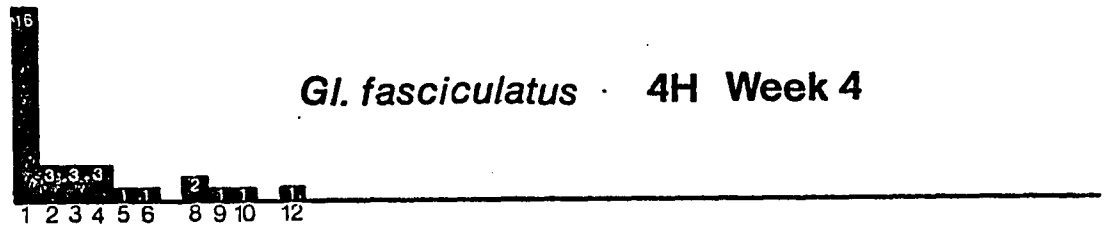
R

E

*Gl. geosporus* 4H Week 0

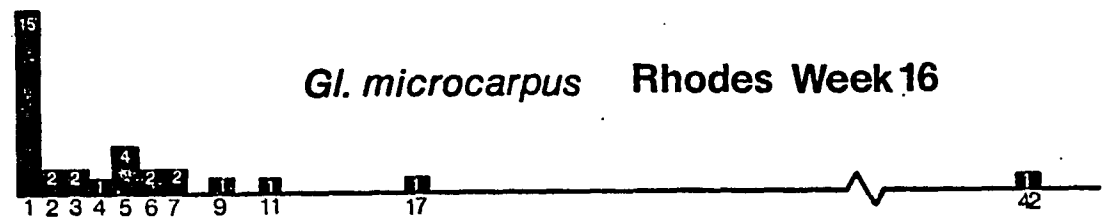
Q

U

*Gl. fasciculatus* 4H Week 4

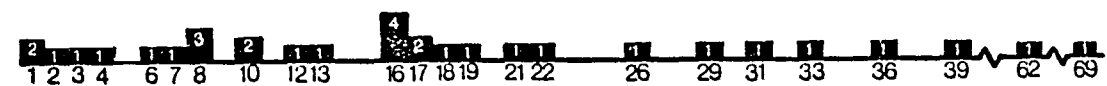
E

N

*Gl. microcarpus* Rhodes Week 16

C

Y

*A. scrobiculata* Rhodes Week 6

C L A S S

total diversity and population levels, even in such small sites as those used for these studies (0.17 ha at 4H and 0.6 ha at Rhodes). Surveys in which only two or three samples are taken will not adequately represent the populations at such locations.

#### Treatment effects

Spore numbers were expressed as spores per kg of oven dry soil. An overall analysis of variance of numbers of spores among treatments, fungal species, and times was performed on the data from each site (Tables 2.2 and 2.3). Conservative degrees of freedom were used for testing the F values to take account of the non-independence imposed by the repeated measures (Morrison, 1967). At Rhodes, Gigaspora gilmorei occurred in such small numbers and with such uneven distribution that it was omitted from this analysis. For the 4H data, because of the missing data, times 2 and 3 were dropped from the analyses.

The results at 4H showed no clonal differences. This was confirmed by other analyses of variance of numbers of spores among treatments for each fungal species at each time. Of the 90 analyses of variance, four showed clonal differences significant at the 0.05 level. However, with this number of comparisons, four or five could be significant purely by chance. Since there were no consistent trends among the differences, they were considered to be Type I errors. We concluded, therefore, that the trees

Table 2.2. Analysis of variance of numbers of spores at the 4H site over all clones, spore species, and times

Source	Degrees of freedom <sup>a</sup>	Mean square	F value
Block (B)	6	205 707	1.93
Clone (C)	3	73 249	0.69
Error a	18	106 694	
Fungal species (S)	8(1)	9 398 705	68.31***
C x S	24(3)	28 329	0.21
Error b	192(24)	137 590	
Time (T)	7(1)	380 239	9.04***
C x T	21(3)	52 019	1.24
Error c	168(24)	42 040	
S x T	56(1)	131 147	4.40*
C x S x T	168(3)	35 348	1.19
Error d	1344(24)	29 798	

<sup>a</sup>Figures in parentheses are conservative degrees of freedom used for testing F values.

\*Significant at  $p \leq 0.05$ .

\*\*\*Significant at  $p \leq 0.001$ .

Table 2.3. Analysis of variance of numbers of spores at the Rhodes site over all treatments, spore species, and times

Source	Degrees of freedom <sup>a</sup>	Mean square	F value
Treatment (Tr)	7	3 091 489	3.70**
Error a	24	835 287	
Spore species (S)	9(1)	32 679 332	102.11***
Tr x S	63(7)	1 055 445	3.30**
Error b	216(24)	320 048	
Time (Ti)	8(1)	7 954 184	19.15***
Tr x Ti	56(7)	492 294	1.18
Error c	192(24)	415 440	
Ti x S	72(1)	2 146 413	10.66**
Tr x Ti x S	504(7)	331 368	1.65
Error d	1728(24)	201 412	

<sup>a</sup>Figures in parentheses are conservative degrees of freedom used for testing F values.

\*\*Significant at  $p \leq 0.01$ .

\*\*\*Significant at  $p \leq 0.001$ .



either had no effect on spore production, or had similar effects. Failure to sample unplanted land during the 4H study precluded determination of which of these interpretations was correct. Despite the herbicide treatment, invasion of weeds was rapid, and the results obtained probably represented spore populations in a disturbed site as vegetation re-establishes.

The analysis of variance for the Rhodes site showed significant differences ( $p \leq 0.01$ ) among treatments, and a significant treatment by species interaction ( $p \leq 0.01$ ). A graphical comparison of spore numbers by treatment and species indicated little treatment effect for most fungal species. However, for Glomus occultus and Gl. albidus, plots without trees seemed to have higher spore populations than plots with trees. This is the probable source of the treatment by species interaction. The graphs also showed the untreated plots averaged higher spore numbers than most treated plots. This is the likely source of the significant treatment effect. Other analyses of variance of numbers of spores among treatments for each fungal species at each time were done. Of the 90 analyses, 16 showed significant differences. Except for one species, these differences were scattered and showed no trends, and are considered to be unrelated to treatments. Glomus albidus, however, showed differences for the samples taken 6(\*\*)<sup>1</sup>, 8(\*\*), 10(\*\*\*), 12(\*\*), and 14(\*) weeks after the planting date. To investigate further

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<sup>1</sup>Asterisks in parentheses indicate levels of significance: \* indicates  $p \leq 0.05$ ; \*\* indicates  $p \leq 0.01$ ; \*\*\* indicates  $p \leq 0.001$ .

treatment differences, two sets of a priori comparisons were made. The first compared the plots containing trees (Canada Blanc vs Eugenii; Tristis #1 vs both euroamerican clones; and Crandon vs both euroamerican clones), and all plots with trees vs P + H plots. These analyses showed that none of the clones had an effect on spore populations. This result supports the opinion that populations of spores at 4H were unaffected by the trees. Examination of the poplar roots at both locations revealed little mycorrhizal development, despite the abundance of inoculum (H. S. McNabb, Jr. Iowa State University; unpublished data). Why this condition existed was not known, but lack of mycorrhizal formation explains the lack of influence on the spore populations.

The second set of comparisons of the Rhodes data considered the 2 x 2 factorial of untreated and treated land (U, P, H, and P + H). The results of these comparisons confirmed the earlier analyses of variance, showing that only for Gl. albidus were differences consistent. The herbicide treatment depressed spore populations ( $p \leq 0.05$ ), although the effects were not strong. Mean populations, in spores per kg of oven dry soil were for week 6, U = 205, H = 31; for week 8, U = 249, H = 117; for week 10, U = 445, H = 127; for week 12, U = 357, H = 112; and for week 14, U = 302, H = 95. Thus, although populations were reduced, there were many spores remaining in the soil, not only of Gl. albidus, but also of other species which apparently were unaffected by the herbicide.

Unless viability of the spores was adversely affected, mycorrhizal establishment should not have been inhibited by lack of spores. P x H interactions were significant only twice ( $p \leq 0.05$ ) out of the 99 analyses, and these occasions are considered to be Type I errors. The lack of interaction indicated that no synergism existed between plowing effects and herbicide treatment.

### Population dynamics

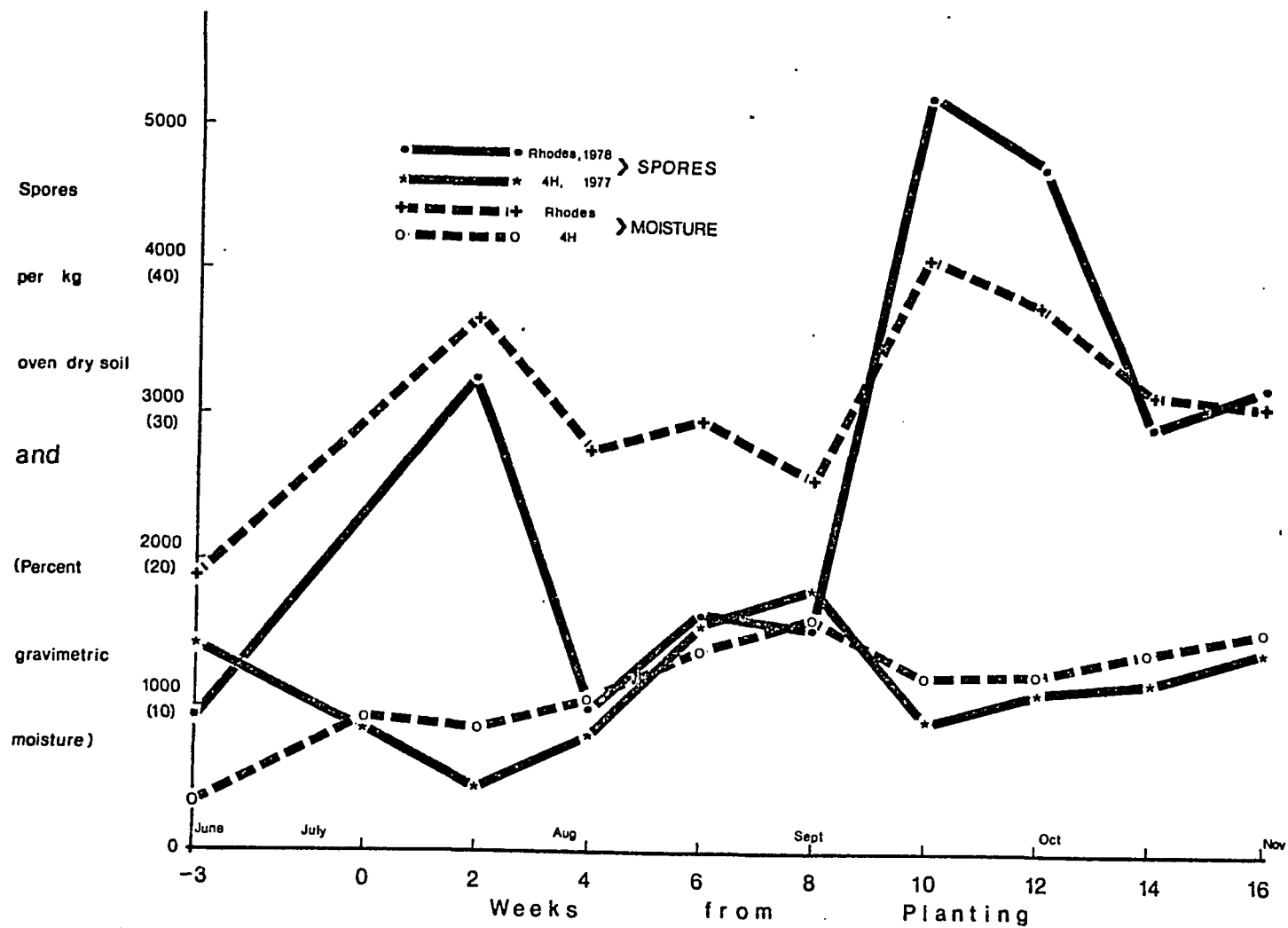
The figures for spore populations were lumped for each time, and the surveys considered as field-studies of endogonaceous spore populations in disturbed soils. The data were handled in this manner because the experiments yielded so few significant differences among clones and treatments. The significant differences for Gl. albidus are not large enough to affect the overall trends. Therefore, all plots and species have been included in calculations of the means.

Examination of total spore populations for both sites showed two population peaks which tended to parallel soil moisture content (Figure 2.4). Mean percentage of moisture and mean spore numbers (all species totalled) are positively correlated at Rhodes ( $r = 0.914^{**}$ )<sup>1</sup>. At the 4H site, the correlation coefficient was only 0.415 (not significant) if all sampling dates were used. But if the initial date was dropped, the correlation became strong, with a correlation coefficient of 0.919<sup>\*\*</sup>. The 1975-77 drought

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<sup>1</sup>Asterisks indicate levels of significance: \*\* indicates  $p \leq 0.05$ ; n = 8 & 9 at 4H, and 10 at Rhodes.

Figure 2.4. Mean endogonaceous spore populations (all species) and mean gravimetric moisture content percentage for two field sites in central Iowa, plotted against sampling times. Time 0 was time of planting poplar clones in some of the plots



was at its peak during the initial sampling period. Because moisture content at the 4H site was only 3.3%, the plants were probably under moisture stress, especially in the upper parts of the soil. Perhaps moisture content is related linearly to spore populations except when some lower threshold of available moisture is reached, after which, germination and spore production cease, and populations remain static. The decrease in spore numbers from July to August at the 4H site may have been a result of spore germination when moisture content rose, prior to renewal of sporulation. A critical examination of moisture effects upon sporulation, germination, and spore mortality should be made.

The relationship of overall levels of spores to moisture was similar to that observed for disturbed sites in Australian heathlands (Sward et al., 1978), although rainfall data were used instead of soil moisture content. The correlation did not occur in nearby undisturbed sites in the Australian study, which prompted comparisons of the untreated plots and treated but unplanted plots at Rhodes. Although mean moisture levels were lower in the untreated than in the treated plots, the changes over time were parallel. Correlating moisture with spore numbers gave correlation coefficients of 0.900\*\* and 0.913\*\* respectively. These values showed that soil and vegetation disturbance did not eliminate moisture effects. Observations in Nigeria, also based on rainfall data, suggested a positive correlation between spore populations and moisture in some sites,

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\*\* Statistically significant correlations:  $p \leq 0.05$ ;  $n = 10$ .

but not in others (Redhead, 1977).

The general trend of populations in the two sites was for numbers of spores to be lowest in spring, and highest in late summer and early autumn. Similar trends have been observed in previous studies, and such trends may be related to root senescence (Khan, 1974; Mason, 1964; Saif, 1977; Sutton & Barron, 1972). A logical assumption, at least for fungi symbiotic with annual plants, is that resting spores are produced as winter approaches.

### Spore numbers

Changes in spore populations over time were very highly significant (Tables 2.2 and 2.3). The species by time interactions in the overall analyses of variance were also statistically significant. This indicated that changes over time were not parallel for all species, and this was confirmed by comparison of the means (Tables 2.4 and 2.5). Nevertheless, there was considerable coincidence of peaks and troughs in population levels for the different species within each location, and sporulation appeared to be influenced by some common factor or set of factors. Whether this was directly linked to moisture, as the overall correlations implied, or was caused by other influences such as root growth or host physiology was unknown.

Population levels for each spore were variable both within and between times. Within a time, individual species had extremely

Table 2.4. Mean spore populations (spores per kg oven dry soil) for each sampling time at the 4H site; values in parenthesis are coefficients of variation (%) (values with question marks are estimates; see text)

Fungal species	Mean spore counts and coefficients of variation at weeks after date of planting poplar clones ( 7 July 1977)			
	-3	0	2	4
<u>Acaulospora</u> <u>scrobiculata</u>	801 (72)	429? ?	241? ?	450 (81)
<u>A. spinosa</u>	80 (86)	26 (231)	2 (219)	96 (79)
<u>Gigaspora</u> spp <sup>a</sup>	194 (170)	141 (146)	62 (112)	86 (106)
<u>Gi. roseae</u> (young)	49 (209)	43 (149)	14 (121)	20 (155)
<u>Gi. heterogama</u>	1 (529)	54 (332)	22 (188)	61 (257)
<u>Glomus</u> <u>constrictus</u>	31 (502)	1 (294)	1 (529)	49 (316)
<u>Gl. fasciculatus</u>	213 (70)	142? ?	83? ?	18 (171)
<u>Gl. geosporus</u>	18 (161)	7 (324)	6 (234)	19 (199)
<u>Gl. mosseae</u>	27 (362)	3 (265)	2 (269)	6 (212)
TOTAL	1416 (56)	846? (50)?	433? (30)?	807 (46)

<sup>a</sup>Gigaspora calospora, Gi. gilmorei, and Gi. rosea (mature), see text.



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6	8	10	12	14	16
971 (66)	785 (43)	462 (67)	621 (95)	533 (102)	713 (68)
213 (57)	224 (58)	91 (79)	127 (105)	104 (78)	119 (56)
208 (166)	275 (159)	127 (119)	92 (107)	152 (143)	148 (168)
41 (149)	27 (118)	26 (108)	22 (115)	34 (126)	40 (112)
14 (301)	89 (232)	51 (243)	25 (270)	82 (151)	47 (236)
32 (351)	44 (385)	4 (445)	4 (529)	19 (455)	14 (364)
64 (99)	251 (66)	83 (152)	176 (74)	201 (68)	223 (72)
35 (199)	126 (244)	40 (221)	37 (208)	64 (147)	88 (139)
20 (192)	17 (233)	10 (185)	7 (214)	3 (236)	12 (169)
1599 (49)	1837 (50)	893 (48)	1110 (62)	1191 (58)	1403 (49)

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Table 2.5. Mean spore populations (spores per kg oven dry soil) for each sampling time at the Rhodes site; values in parenthesis are coefficients of variation (%)

Fungal species <sup>a</sup>	Mean spore counts and coefficients of variation at weeks after date of planting poplar clones (12 July, 1978)			
	-3	2	4	6
<u>Acaulospora</u> <u>scrobiculata</u>	52 (110)	570 (187)	115 (161)	180 (89)
<u>A. trappei</u>	123 (77)	856 (95)	391 (94)	632 (69)
<u>Gigaspora</u> <u>calospora</u>	303 (175)	129 (116)	105 (77)	182 (79)
<u>Gi. gilmorei</u>	10 (240)	12 (202)	3 (443)	1 (316)
<u>Glomus</u> <u>albidus</u>	78 (138)	483 (237)	46 (170)	72 (113)
<u>Gl. epigaeus</u>	46 (98)	84 (170)	13 (212)	13 (169)
<u>Gl. fasciculatus</u>	41 (204)	147 (167)	63 (281)	51 (142)
<u>Gl. geosporus</u>	7 (288)	46 (225)	30 (205)	26 (192)
<u>Gl. microcarpus</u>	43 (217)	163 (100)	12 (277)	48 (192)
<u>Gl. mosseae</u>	58 (131)	181 (141)	34 (222)	22 (261)
<u>Gl. occultus</u>	164 (72)	626 (101)	209 (113)	412 (90)
TOTAL	925 (79)	3298 (92)	1021 (71)	1641 (55)

<sup>a</sup>Acaulospora spinosa not counted, see text.

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8	10	12	14	16
246 (139)	848 (126)	701 (172)	513 (108)	250 (115)
441 (100)	1660 (70)	1554 (70)	900 (90)	977 (91)
144 (103)	214 (111)	229 (128)	152 (129)	173 (111)
1 (318)	5 (345)	1 (316)	4 (257)	11 (290)
82 (117)	116 (155)	107 (145)	94 (145)	43 (98)
15 (199)	28 (172)	21 (346)	16 (219)	10 (245)
20 (133)	67 (169)	80 (223)	33 (132)	53 (155)
30 (259)	50 (130)	45 (131)	57 (205)	35 (181)
34 (145)	155 (209)	170 (166)	99 (124)	41 (193)
12 (145)	127 (151)	111 (157)	36 (179)	65 (145)
539 (128)	1990 (77)	1748 (145)	1073 (106)	1548 (104)
1563 (82)	5261 (62)	4767 (76)	2975 (87)	3206 (70)

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high variances, often highest for species present in very low numbers (Tables 2.4 and 2.5). The range of values (in spores per kg oven dry soil) over all times at Rhodes was 144 to 16 612 (mean 2740; CV 99%), and at 4H the range was 86-4479 (mean 1153; CV 64%). The range of sample means (all species) at Rhodes was 925-5261, and at 4H, 433-1873. These figures are comparable to those reported by some other workers (Hayman, 1978; Koske, 1975; Thapar & Khan, 1973), but lower than the maximum of 51 580 spores per kg (moist soil) reported by Saif & Iffat (1976), or the 400-62 000 spores per liter of soil shown by Powell (1977). However, these last two studies used small amounts of soil (50 g and 5 ml, respectively) and an agitation-settling technique for spore extraction. This extraction method may be more efficient than the bulk extractions done in other studies.

The poor mycorrhizal development in the poplar clones is puzzling, considering the large number of endogonaceous spores in the rhizosphere. All the poplar clones used form endomycorrhizae with members of the Endogonaceae. Indeed, one clone, Canada Blanc, so readily becomes mycorrhizal that it is used as a host for pot cultures of Endogonaceae at Iowa State University. At first, we thought that drought conditions at the 4H site may have affected adversely mycorrhizal colonization, but no shortage of moisture existed in 1978, and establishment of mycorrhizae was not improved at Rhodes.

## CONCLUSIONS

The two sites had more diverse populations of endogonaceous fungi than those reported in previous studies. This large diversity may not be unusual. Frequently, extraction of spores has been done with sieves having openings no smaller than 100  $\mu\text{m}$  (Ames & Linderman, 1977), and small-spored species such as found in these studies may have been overlooked.

Despite large populations of spores, the poplars rarely became mycorrhizal, and these plants had no effects on spore populations. Changes in spore numbers did, however, take place over time. These changes apparently were related to soil moisture level. Whether this relationship was direct or indirect could not be deduced from these studies.

A slight indication was found that plowing or application of herbicides could depress spore production. In only one fungal species (Glomus albidus) was this trend statistically significant. Further studies on effects of site preparation on populations of endogonaceous spores would be worthwhile.

The distribution of species and spores within the soil was extremely variable, depending partially upon fungal species. For example, Gl. constrictus at the 4H site occurred in discrete parts of the area. In the plots where it existed, this fungus was not recorded at every sampling date. Acaulospora spinosa was common

and abundant over both space and time at the 4H site, but it was so rare at the Rhodes site that it was excluded from the counts. Examination of the distribution of native vegetation and the location of roots in the soil might explain the clumped distributions. Perhaps host preference exists for some species but not for others. Similar disparity was observed in grassland sites in western North America (Molina et al., 1978) Thorough ecological surveys would clarify many of these observations.

The diverse, disparate, and highly variable spore populations shown in these surveys, clearly prove that small samples with but few replicates taken at only one time, will not represent adequately the populations of even a small area of land.

## ACKNOWLEDGMENTS

We wish to thank Deborah A. Kvach Ketcham and Kathleen M. Bason for their technical assistance, and Jim Neppel, Manager, 4H Camping Center, Boone Co., Iowa for his help in allowing the use of the 4H site and in transporting water to the trees. Thanks also to William F. Haywood, Thomas L. Green, and Terry L. Robison for cheerfully volunteering assistance in erecting a deer-fence around the Rhodes plots.

Dr. Don C. Norton, Professor of of Plant Pathology, Iowa State University, gave us details of the centrifugation sugar-flotation method that we used for extracting spores from the soil.

We would like to thank Dr. Paul N. Hinz, Professor of Statistics, Iowa State University, for his advanced statistical advice.

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## PART 3.

COMPLEXIPES MONILIFORMIS: A NEW GENUS AND SPECIES

TENTATIVELY PLACED IN THE ENDOGONACEAE

Complexipes moniliiformis: A new genus and species  
tentatively placed in the Endogonaceae

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## INTRODUCTION

While investigating the Endogonaceae of Iowa , I sampled soil from the Ames High School Pinewood, Ames, Iowa. This area is a small plantation of red pine (Pinus resinosa Ait.), Scots pine (P. sylvestris L.), and eastern white pine (P. strobus L.) about 35 years old. The ground cover consists of virginia creeper (Parthenocissus quinquefolia (L.) Planch.) in a dense monospecific mat. The soil was sampled for endogonaceous spores with a centrifugation and sugar-flotation technique similar to that of Jenkins (1964). Glomus fasciculatus (Thaxter sensu Gerdemann) Gerdemann & Trappe and G. etunicatus Becker & Gerdemann were present in great abundance, along with another rather ornate spore, which is here named and placed in the new genus Complexipes. Specimens have been deposited in the Oregon State University Herbarium (OSC). In addition, I have retained some in my personal collection (Walker #27).

COMPLEXIPES MONILIFORMIS Walker gen. et sp. nov.

(Figure 1)

[LATIN DIAGNOSIS]<sup>1</sup>

Sporocarps unknown. Chlamydospores borne singly in the soil, terminal on a single hyphal attachment, globose, 55-110  $\mu\text{m}$  in diam, orange-brown to dark red-brown. Spore wall three-layered, with an outer layer 0.5-1  $\mu\text{m}$  thick fused to a middle layer up to 6  $\mu\text{m}$  thick and an inner layer 0.5-1  $\mu\text{m}$  thick. Outer layer often difficult to distinguish even in crushed specimens, ornamented with crowded folds and papillae up to 4  $\mu\text{m}$  high.

Subtending hypha with a thick 2-layered wall and up to nine cells formed by septa derived from the inner wall, the cells slightly constricted at the septa and readily detaching from the spore base to leave a light-colored circular scar; hyaline to pale yellow, the subtending cell usually brown tinted, papillate to occasionally rugose, the ornamentation less dense than on the spore. Subtending cell usually cup-shaped, up to 24 x 24  $\mu\text{m}$ .

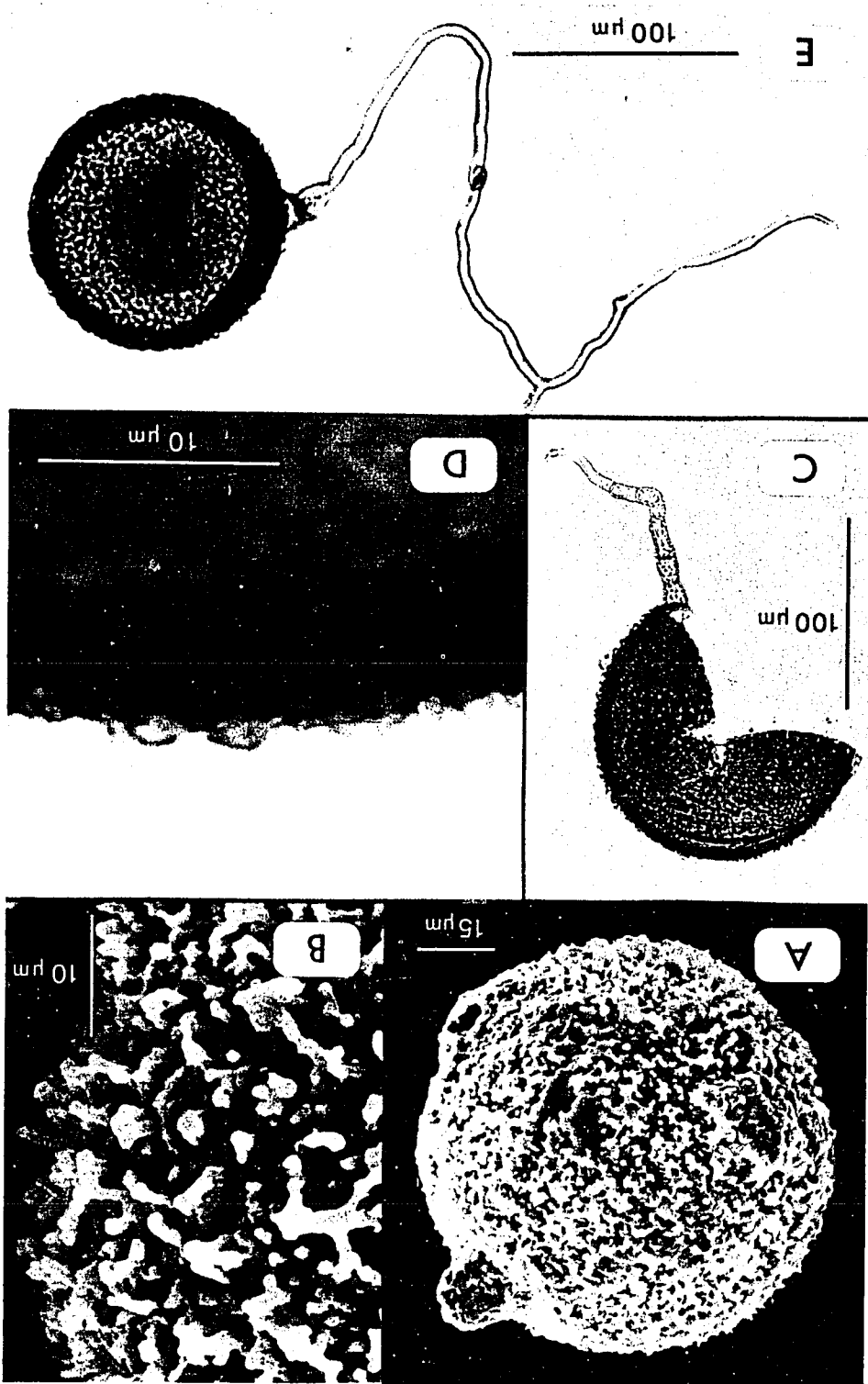
Hyphae in the soil pale yellow, thick-walled, sparsely low rugose, coenocytic, up to 6  $\mu\text{m}$  in diam, the walls up to 1  $\mu\text{m}$  thick.

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<sup>1</sup>Latin diagnosis omitted so that this does not constitute a legal description under the rules of the International Nomenclature Committee.

Figure 3.1. Complexipes moniliiformis

- (A) Scanning electron micrograph of a mature spore showing cupulate suspensor-cell.
- (B) Detail of complex outer wall coating.
- (C) Light micrograph of a mature spore showing moniliiform subtending hypha.
- (D) Detail of wall structure.
- (E) Mature spore attached to coarse non-septate hypha.





TYPE: Ames, Iowa, U.S.A., Walker #27 (OSC).

### Distribution and habitat

In soils around roots of virginia creeper (Parthenocissus quinquefolia) in a pine plantation. Mosse & Bowen (1968) described a fungus that probably is C. moniliformis as a "crenulate spore" from soils in New Zealand and Australia, but they gave no details of associated plants. Thapar & Khan (1973) also recorded and figured a similar spore from their survey of some Indian soils. Their records were from forest nurseries on grassland soils in Kerala State, but host species were not identified. Hall (1977) discovered a similar species in a disused garden in New Zealand and, in the same publication, cited Dr. Barbara Mosse as having found it in Germany. Wilcox et al. (1974) describe and excellently illustrate this fungus (as BDG-58) in detail and also refer to Mikola's "E-57" and "E strain" as the same species. Both "BDG-58" and the "E" fungi were associated with the roots of pine trees.

### Mycorrhizal associations

Forms ectendomycorrhizae with pines (Wilcox et al., 1974). Associated in the field at the type location with mycorrhizal roots of pines and virginia creeper.

### Etymology

Genus - Latin, "complex base" referring to the complex form of

the subtending hypha.

Species - Latin, "necklace-like", also referring to the subtending hypha.

## DISCUSSION

Complexipes differs from other genera in the Endogonaceae by having a moniliiform subtending hypha and a highly ornamented outer coat, which seemingly is secreted by the middle wall layer and becomes more complex with maturity. Gigaspora species have azygospores produced on a bulbous suspensor cell, which often is formed terminally on a septate hypha. Complexipes chlamydospores are borne on a cupulate cell and, whereas the hyphae of Gigaspora do not break readily at the septa, the moniliiform cells of Complexipes are readily separable. Individual Glomus and Sclerocystis chlamydospores have some similarities with those of Complexipes, but do not have the cup-shaped suspensor cell and moniliiform subtending hypha. When detached from their subtending hypha, Complexipes spores could be mistaken for similarly detached spores of Gigaspora heterogama (Nicol. & Gerd.) Gerdemann & Trappe or for Acaulospora spores. Care should be taken to see that sessile spores in the Endogonaceae are correctly identified. Fortunately, numerous spores are generally present in a soil sample, and careful comparison of all spores from the sample usually will lead correct conclusions.

Wilcox and his co-workers have shown that C. moniliiformis is culturable and has septate hyphae. However, the small amount of hypha found in my field collections is but sparsely septate (Figure 3.1E) and is coarse and thick-walled. It appears more akin to the coenocytic hyphae of the Endogonaceae than to the more regularly septate hyphae of most ascomycetes. Hall (1977) suggested that it might be an ascomycete, but in the same paper, he described the hyphae

as being aseptate. As far as I can judge from the plates in Wilcox et al., some lengths of hyphae in his cultures were aseptate. Septa are not uncommon in hyphae of the Endogonaceae.

That the BDG-58 fungus of Wilcox et al. was ectendomycorrhizal rather than endomycorrhizal does not preclude its membership in the Endogonaceae. Members of the family form endo- and ectomycorrhizae, some species are seemingly saprobic, and the mycorrhizal relationships of yet others are unknown (Gerdemann & Trappe, 1974). The fungus sporulated only when associated with plant roots (Wilcox et al.). This also is in line with the behavior of the culturable Endogone eucalypti nom. ined. (Warcup, 1975). In addition, the mycorrhizae formed by the BDG-58 fungus (as illustrated by Wilcox et al.) are not altogether unlike those formed by Glomus or Gigaspora under some circumstances, despite the authors' comments to the contrary. The intracellular structures could certainly be considered as arbuscular in general appearance, albeit somewhat coarse. The "Hartig net" is analogous in some ways to the often highly septate intercellular hyphae of vesicular-arbuscular mycorrhizae. The description of the sheath suggests an apparent similarity to that formed on Eucalyptus by Endogone eucalypti.

I have concluded, therefore, that Complexipes sufficiently resembles fungi already placed in the Endogonaceae to be provisionally placed as a new genus in that family.

## ACKNOWLEDGMENTS

I wish to thank Dr. James M. Trappe for his helpful comments, for preparing the Latin diagnosis, and for suggesting the generic and specific names.

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## PART 4.

ACAULOSPORA SPINOSA: A NEW SPECIES IN THE ENDOGONACEAE

Acaulospora spinosa: A new species in the Endogonaceae

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## INTRODUCTION

Surveys of the Endogonaceae in Mexico by Trappe and Iowa by Walker led to the discovery of an undescribed taxon in the genus Acaulospora. The species is easily distinguished from all other named members of the genus (Ames & Linderman, 1976; Gerdemann & Trappe, 1974; Nicolson & Schenck, 1979; Rothwell & Trappe, 1979; Trappe, 1977) and is extremely common in the type locality, a field site in central Iowa.

ACAULOSPORA SPINOSA Walker & Trappe sp. nov.

(Figures 4.1 and 4.2)

[LATIN DIAGNOSIS]<sup>1</sup>

Sporocarps unknown. Azygospores formed singly in the soil, sessile, attached by an 8-15  $\mu\text{m}$  broad collar to the side of a funnel-shaped to cylindrical hypha, which sometimes has thin tapering hyphal projections and terminates in a globose vesicle about the same size as the spore. Vesicle becoming empty and shrunken at spore maturity. Vesicle and hypha with hyaline to yellow walls 0.5-3  $\mu\text{m}$  thick. Spores 100-298 x 100-335  $\mu\text{m}$ , usually globose to subglobose, but some specimens ellipsoid or reniform; dull beige to dark red-brown, usually with at least part of the vesicle remaining attached. Surface ornamented with crowded blunt polygonal spines 1-4  $\mu\text{m}$  high, 1  $\mu\text{m}$  wide at base, tapering to 0.5  $\mu\text{m}$  at the tip. Separated by about 0.2  $\mu\text{m}$  to give a reticulate appearance through a compound microscope when focused on base. Developing crusted patches of a hyaline to subhyaline material superimposed upon the spines. Crusted patches rarely covering the whole spore surface, up to 2  $\mu\text{m}$  thick. Spore wall continuous except for the occluded opening: three-layered, the outer layer beige

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<sup>1</sup>Latin diagnosis omitted so that this does not constitute a legal description under the rules of the International Nomenclature Committee.

Figure 4.1. Acaulospora spinosa azygospores.

(A) Scanning electron micrograph (SEM) showing globose form, surface ornamentation, and the occluded pore (arrowed).

(B) SEM showing portion of mother vesicle still adhering to the spore and the cerebriform convoluted encrustations on the spore surface.

(C) SEM detail of the dense crowded spines with patches of encrustation.

(D) Azygospore attached to collapsed mother vesicle (arrowed).

(E) A somewhat reniform spore. Part of the mother vesicle and the occluded pore can be seen (arrowed).

(F) Detail of wall structure. The three walls are arrowed.

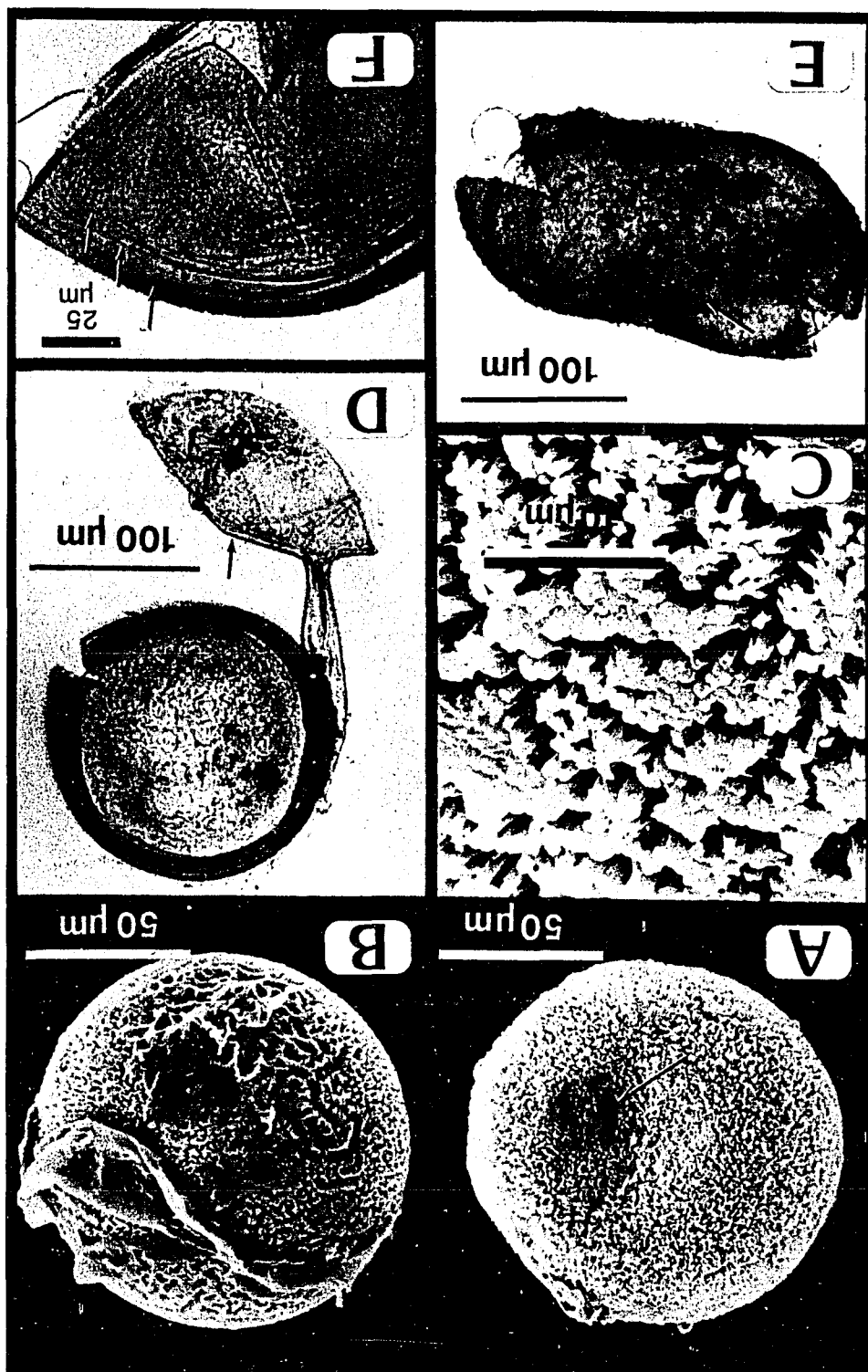


Figure 4.2. Detailed light micrographs of outer wall surface of Acaulospora spinosa

(A) Cracked spore showing details of spines, Tips (t) and bases with reticulate appearance (b) can be seen.

(B) Lower magnification detail of tips of spines, showing a somewhat swirled appearance.

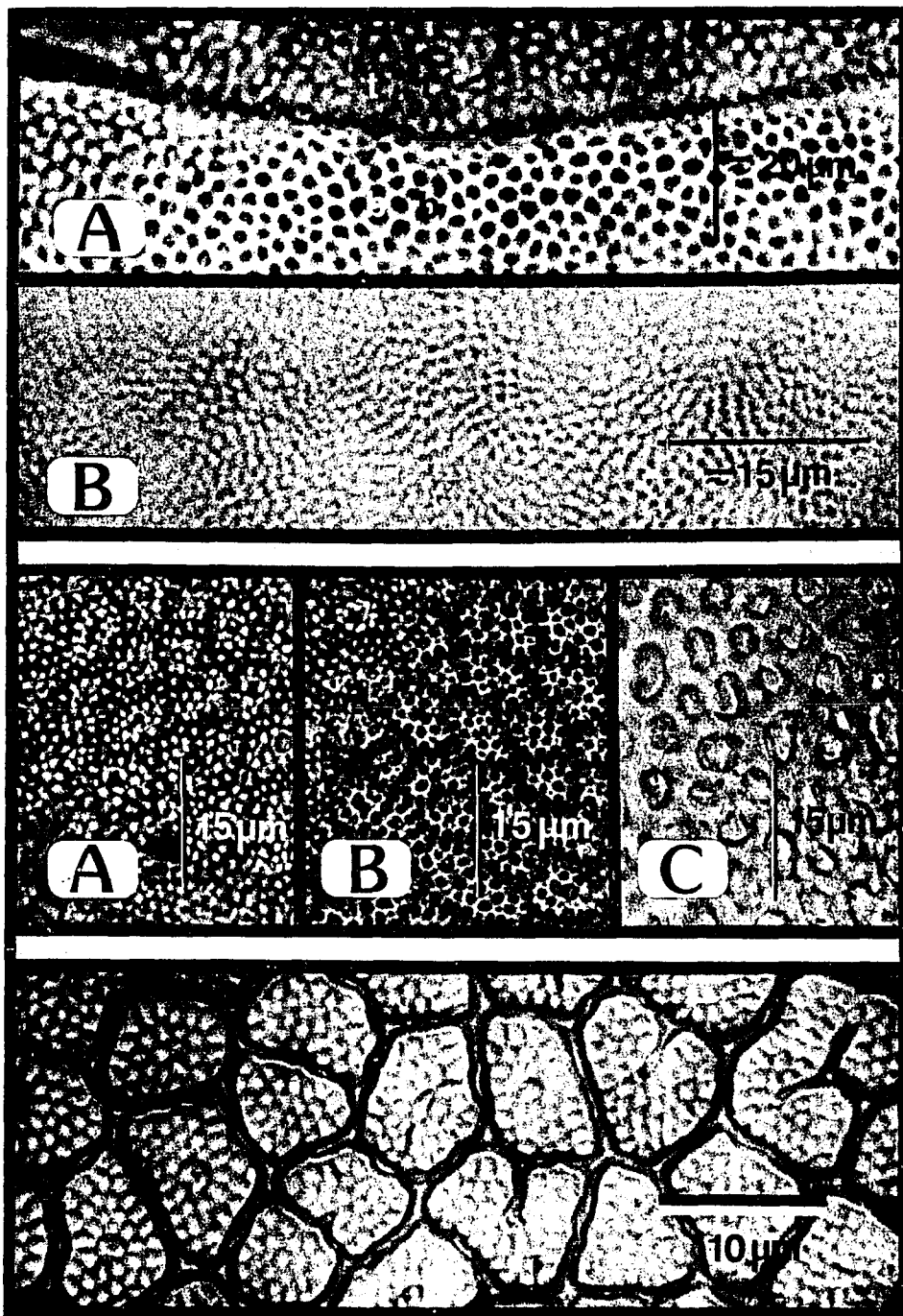
Figure 4.3. Detailed light micrographs of outer wall surface of Acaulospora elegans

(A) Microscope focused on tips of spines.

(B) Microscope focused on bases of spines to show reticulate appearance.

(C) The reticulum overlaying the spines. Tips of the spines can be seen out-of-focus in the alveoli.

Figure 4.4. Detailed light micrographs of outer wall surface of Acaulospora bireticulata showing the double reticulum (photograph by F. M. Rothwell, from Rothwell & Trappe, 1979).



to red-brown, 4-10  $\mu\text{m}$  thick (including spines and encrustations) enclosing 2 membranous hyaline walls, each 0.2-1  $\mu\text{m}$  thick, the inner wall usually slightly thinner.

#### Distribution and habitat

Known from a sandy river terrace near the Des Moines River in central Iowa, where it is abundant throughout the growing season in the soil around the roots of annual grasses, forbs, and trees. Also found in much smaller numbers in a heavy black soil in an old meadow site near Rhodes, Iowa. In both these sites, it is associated with other endomycorrhizal Endogonaceae in the genera Acaulospora, Gigaspora, and Glomus. Found in Mexico in the soil beneath roadside grasses and weeds.

#### Mycorrhizal associations

Forms vesicular-arbuscular mycorrhizae with strawberry (Fragaria vesca L.). Associated in the field with mycorrhizal roots of grasses, forbs, and trees (Populus spp. and Fraxinus americana L.) in Iowa and of roadside grasses and weeds in Mexico.

#### Etymology

Latin, spinosa; referring to the crowded spines covering the outer wall.

Collections examined

TYPE: U.S.A., IOWA, Boone County, 4H Camp (Walker #164) (OSC, Isotype FH, ISC).

PARATYPE: MEXICO, Veracruz, Tuitla Biological Field Station, Univ. Nac. Auton. Mexico, Municipio San Andres (Trappe #3596) (OSC). The two collections differ slightly. The spores from Iowa are lighter in color and more beige; those from Mexico more often are deep red-brown. The Mexican spores are, in general, somewhat larger, though their ranges overlap considerably. The differences are not sufficient to propose two separate taxa.



## DISCUSSION

Acaulospora spinosa can be distinguished from most presently described members of the genus by the ornamentation of crowded spines on the surface of its outer wall. Acaulospora elegans Trappe & Gerdemann and A. bireticulata Rothwell & Trappe both have highly ornamented surfaces (Figures 4.3 and 4.4), but the reticulum found on the former and the double reticulum on the latter are easily observed. Occasional spores of A. spinosa have the outer crust formed into cerebriform convolutions (Figure 4.1B), which are suggestive of a reticulum and may indicate an evolutionary connexion between these three species. However, the convolutions are evident only under the scanning electron microscope and cannot be seen by light microscopy. Young spores of A. elegans may not have their reticulum developed fully (Gerdemann & Trappe, 1974), but it is unlikely that a collection would lack mature specimens. As with all fungi, identifications are best made from a series of mature specimens.

The widely spaced localities from which this species has been found lead to the suggestion that it may be found in other places in Continental America.

KEY TO THE SPECIES OF ACAULOSPORA

- 1 Spores less than 100  $\mu\text{m}$  in diameter, globose to ellipsoid or obovoid. Hyaline to slightly yellow. Wall seemingly single. Surface ornamentation difficult or impossible to see under a compound light microscope.....A. trappei
- 1 Spores larger than 100  $\mu\text{m}$  in diameter. Globose to subglobose; reniform to ellipsoid. Wall of more than one layer.....2
- 2(1) Surface smooth. Globose to subglobose, 119-300 x 119-520  $\mu\text{m}$ . Yellow-brown to red-brown. Wall 3-layered. Outer layer rigid, 2-4  $\mu\text{m}$  thick. Middle and inner layers thin and membranous.....A. laevis
- 2 Surface ornamented with ridges, bumps, hollows, or spines...3
- 3(2) Surface ornamented with spines, with or without a reticulum overlaying the outer wall. Spores globose to subglobose, sometimes ellipsoid or reniform.....4
- 3 Surface ornamented with convoluted ridges or hollows. Spores globose to broadly ellipsoid.....6
- 4(3) Surface ornamented with crowded spines 0.5-2  $\mu\text{m}$  tall. Patches of hyaline encrustations overlaying some spines (observable only in optical cross section), but no reticulum evident.....A. spinosa
- 4 Surface ornamented with crowded spines or processes. At least one reticulum evident on outer surface, though it may be missing in patches, especially on young spores.....5
- 5(4) Surface ornamented with dense spines up to 2  $\mu\text{m}$  tall, over which is laid at maturity a single alveolate reticulum, the ridges approximately 1  $\mu\text{m}$  wide and about 5-6  $\mu\text{m}$  between branches. Spores 140-285 x 145-330  $\mu\text{m}$ .....A. elegans
- 5 Surface ornamented with round-tipped polygonal processes about 1 x 1  $\mu\text{m}$ , overlain by a polygonal reticulum which has a central depression along the ridges. Spores globose, 150-155  $\mu\text{m}$ ; grayish-green to grayish-brown A. bireticulata

- 6(3) Spores dark red-brown, opaque, sometimes almost black; globose to subglobose, 200-260 x 220-260  $\mu\text{m}$ . Outer wall decorated with large depressions 4-8 x 4-16  $\mu\text{m}$ , irregularly separated by 1-12  $\mu\text{m}$ .....A. foveolata Trappe sp. ined.
- 6 Spores white to dull brown. Surface ornamented with hollows and ridges or cerebriform folds. Walls easily separable on crushing.....7
- 7(6) Spores globose, 200-250  $\mu\text{m}$  in diameter. Walls double; outer wall covered with cerebriform folds up to 12  $\mu\text{m}$  tall. Inner wall with an alveolate reticulum formed from ridges separating hollows.....A. gerdemannii
- 7 Spores globose to broadly ellipsoid, 100-240 x 100-220  $\mu\text{m}$ . White to light olive-brown. Surface with depressions 1-1.5 x 1-3  $\mu\text{m}$ , separated by ridges 2-4  $\mu\text{m}$  wide. Spore having the appearance of a miniature golf ball under a compound microscope. Wall 4-layered, but often only an outer and inner layer easy to distinguish.....A. scrobiculata

## ACKNOWLEDGMENTS

Thanks are due to Robert N. Ames, University of California, Berkeley, who established A. spinosa in pot culture with strawberry after attempts at Iowa State University had failed, to Joan Zito and Kathleen Bason, Iowa State University, who prepared the photographic plates of A. spinosa and A. elegans, and to Ned Klopfenstein, who assisted in the scanning electron microscope studies. Thanks also to Dr. F. M. Rothwell, U.S. Forest Service, Berea, Ky., who kindly allowed us to use his photograph of the wall of A. bireticulata.

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## PART 5.

SPECIES IN THE ENDOGONACEAE :

GLOMUS OCCULTUS sp. nov.

AND

GLOMUS GEOSPORUS stat. nov.

## Species in the Endogonaceae:

Glomus occultus sp. nov. and Glomus geosporus stat. nov.

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Studies on black walnut mycorrhizae are supported by Grant-in-aid from the North Central Forest Experiment Station, St. Paul, Minnesota.

Prepared for submission to Mycotaxon.

## DESCRIPTION OF THE NEW SPECIES

During a study of the population dynamics of endogonaceous spores in a field site in central Iowa, a species of Glomus with white to hyaline spores was found in great abundance. Later, I found it contaminating pot cultures of Glomus and Gigaspora spp. in a greenhouse at Oregon State University. It was also found by Denise Fardelmann, Forestry Department, Iowa State University (pers. comm.) from a black walnut (Juglans nigra L.) plantation in Illinois.

Comparison of this entity with the presently described species in the genus (Becker & Gerdemann, 1977; Daniels & Trappe, 1979; Gerdemann & Trappe, 1974; Hall, 1977; Nicolson & Schenck, 1979; Tandy, 1975; Trappe, 1977) showed the fungus to be undescribed, and it is here named Glomus occultus.

Glomus occultus Walker sp. nov. (Figures 5.1 and 5.2)

[LATIN DIAGNOSIS]<sup>1</sup>

Sporocarps unknown. Chlamydospores borne singly or in loose clusters in the soil, or in compact clusters in the cortex of roots, often broader than long, ovoid to obovoid, subangular to irregular, less frequently, globose to subglobose, 35-100 x 40-120  $\mu$ m (mean 80 x 92), hyaline to white. Cyanophilous in cotton blue. Not reacting to Melzer's reagent.

<sup>1</sup>Latin diagnosis omitted so that this does not constitute a legal description under the rules of the International Nomenclature Committee.



Figure 5.1. Chlamydospores of Glomus occultus

(A) A subangular spore with a recurved subtending hypha (arrowed).

(B) and (C) Spores showing the outer coat in the process of sloughing (arrowed).

(D) A spore showing the broader-than-long nature of many specimens.

(E) Spore broken open to show the outer wall separated from the inner wall.

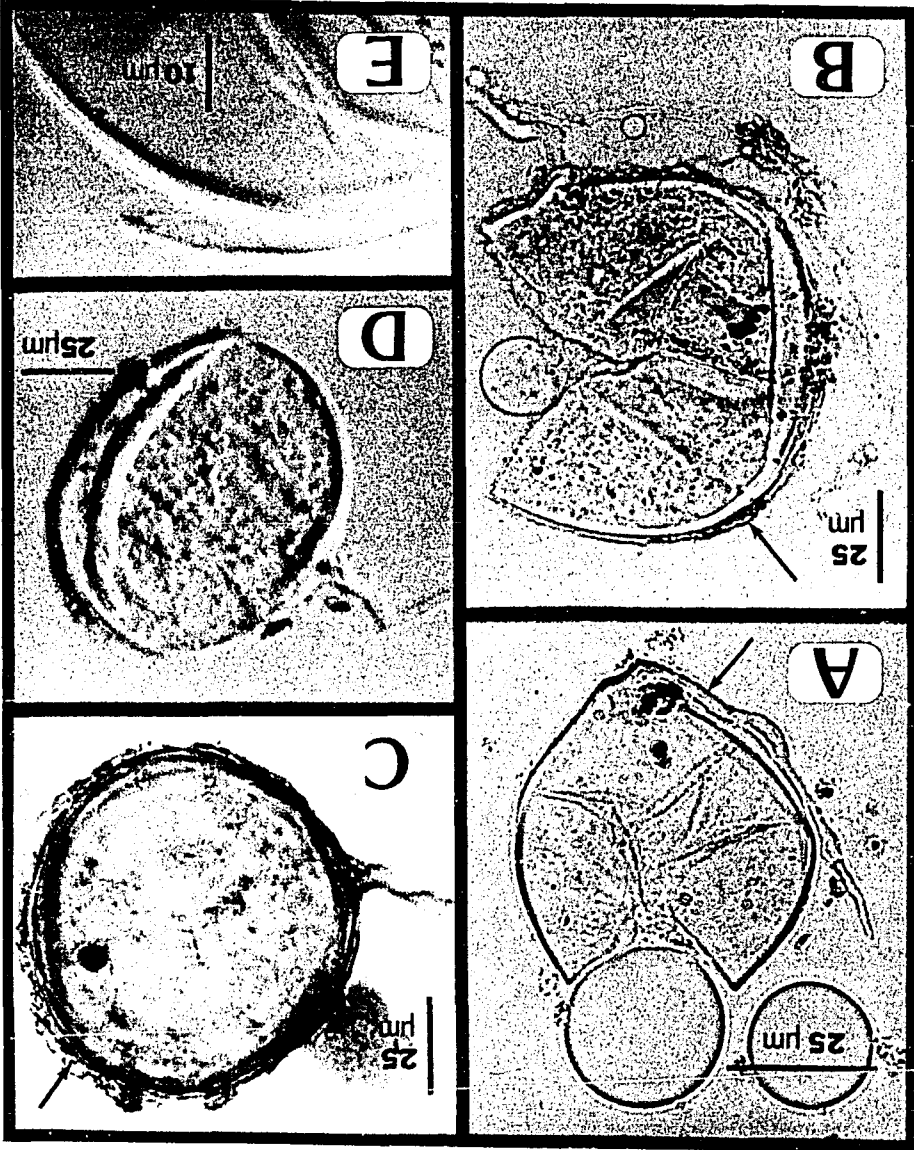
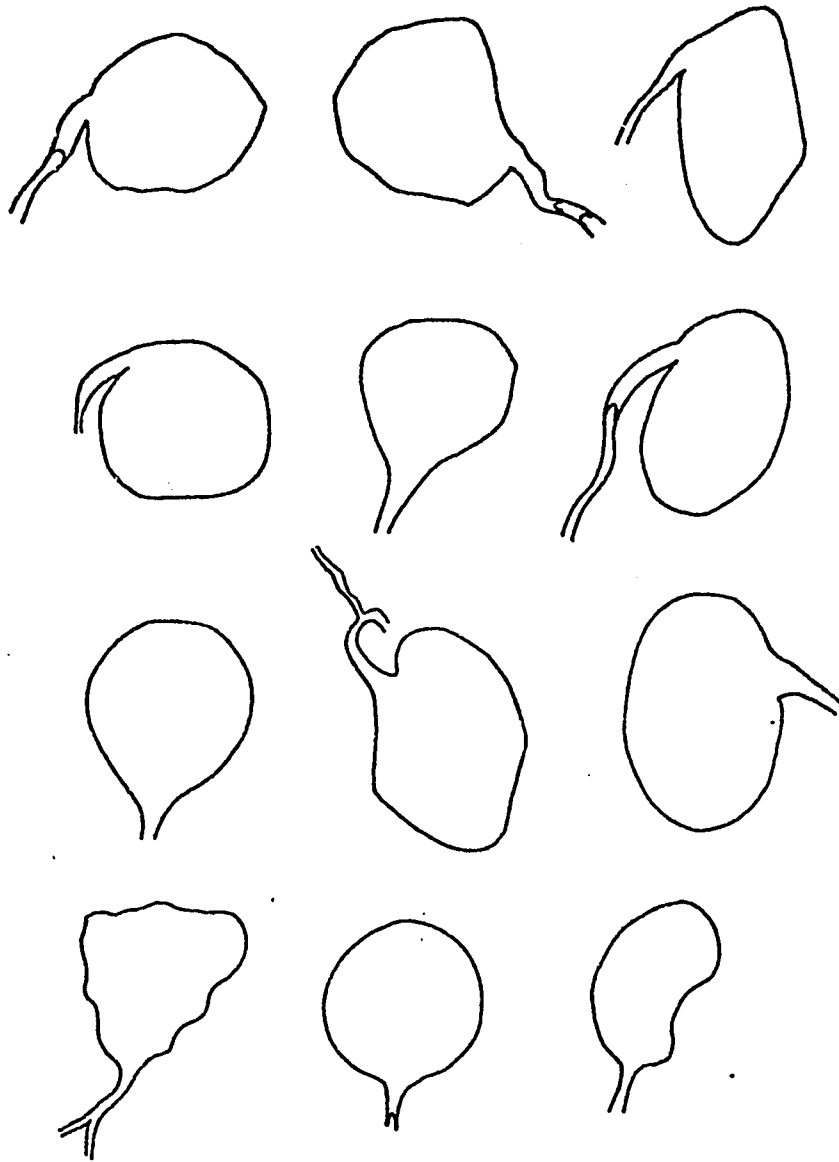


Figure 5.2. Examples of the different shapes taken by spores of Glomus occultus. Note the subangular nature and eccentrically connected subtending hypha in some specimens



NOT TO SCALE

Subtending hypha funnel-shaped to simple, 5-50  $\mu\text{m}$  long, 5-10  $\mu\text{m}$  wide at spore base, tapering to 2-5  $\mu\text{m}$ ; attached axially or eccentrically and recurved to straight, sometimes closed distally by a septum.

Spore wall 2-layered with an additional rough outer deposit of granular material which sloughs with age. Outer deposit up to 2  $\mu\text{m}$  thick except at the spore base where it may be greatly thickened. Outer wall less than 1  $\mu\text{m}$  thick, often indistinct. Inner wall (1-)1.5-2.5(-5)  $\mu\text{m}$  thick, of two sometimes indistinct laminations.

Spore contents enclosed by a thin slightly granular inner membrane which sometimes protrudes into the subtending hypha to give the appearance of a septum.

#### Distribution and habitat

Hypogeous in an old meadow site in Iowa, from pot cultures in Oregon (origin unknown), and from the rhizosphere of a walnut plantation in Illinois. In Iowa, abundant throughout the growing season and probably also as overwintering chlamydospores in the soil.

#### Mycorrhizal associations

Forms vesicular-arbuscular mycorrhizae with Populus x euroamericana (Dode) Guinier and Sorghum vulgare Pers. in pot

culture. Associated in the field with mycorrhizal roots of poplars (Populus spp.), of foxtail grasses (Setaria spp.), of smooth brome (Bromus inermis Leyss.), and of black walnut (Juglans nigra).

### Etymology

Latin, occultus; hidden. Referring to the obscurity of the spores caused by their small size, angular shape, and lack of coloration, which makes them resemble sand grains.

### Collections examined

TYPE: Iowa -- Marshall Co., Rhodes, Iowa State University Rhodes Farm, among roots of grasses and poplars, Walker #93, 1.xi.1978 (OSC; isotype ISC). PARATYPES: OREGON -- Benton Co., Corvallis, Ornamentals Plant Research Laboratory Greenhouse, Oregon State University, Walker #104 (OSC). ILLINOIS -- Jackson Co., Southern Illinois University Progeny Test Center (Fardelmann 015C; collection of D. Fardelmann). In addition, specimens from the Rhodes Farm were examined from random soil samples taken every two weeks during the summer and early autumn of 1978. A pot culture has been established in the Forestry Greenhouse, Iowa State University (culture #274), and spores from this have been deposited in the Herbarium at Oregon State University (OSC).

DISCUSSION OF GLOMUS OCCULTUS

Glomus occultus is distinguished from most other described in the genus by its lack of color and its often subangular outline (Figure 5.2). Gl. pallidus Hall and Gl. clarus Nicolson & Schenck also have hyaline ectocarpic spores (Hall, 1977; Nicolson & Schenck, 1979), but the former is smaller (32-78 x 28-68 compared with 35-100 x 40-120  $\mu\text{m}$ ), and has a simpler wall structure consisting of a single laminated wall. The inner wall of Gl. occultus is bilaminate, though sometimes the laminations are difficult to detect. I have not observed more than two laminations in examination of numerous specimens of Gl. occultus. Gl. clarus spores are larger (290  $\mu\text{m}$  maximum diameter) and the spore walls yellow with age, a rare event with Gl. occultus. The walls of these two species are reversed in relative thickness, i.e., for Gl. clarus, the outer wall is the thicker, while for Gl. occultus, the outer wall is thin and sometimes difficult or impossible to see. Gl. clarus does sometimes have an outer mucilaginous coat which is described as becoming verrucose and folded with age, with folds up to 5  $\mu\text{m}$  in height. The outer covering occurs on all young spores examined in Gl. occultus, and sloughs off with age without folding or becoming verrucose. No such outer wall is mentioned in the description of Gl. pallidus. The subtending hypha of spores of Gl. clarus may extend 400  $\mu\text{m}$  below the spore, and is closed by a bulging septum in the pore. The subtending hypha of Gl. occultus

rarely is longer than 100  $\mu\text{m}$  (often less than 25  $\mu\text{m}$ ), and when a septum exists, it is some distance distad of the spore base.

Spores of Gl. occultus could be easily overlooked in soil sievings. Smallness, lack of color, and an often subangular form make the spores rather difficult to distinguish from sand grains. I have found Gl. occultus spores to be more easily seen if, rather than using the combination of incident and transmitted light recommended by Mosse & Bowen (1968), I use only incident illumination when picking through sievings under the dissecting microscope.

Sieves with openings no less than 100  $\mu\text{m}$  often are used for wet-sieving and decanting endogonaceous spores. Most Gl. occultus spores are smaller than 100  $\mu\text{m}$  and will be lost through such sieves. In view of the wide distribution of this species, it may be common but overlooked in the past.



## THE NEW STATUS

Another Glomus sp. found in Iowa is Gl. macrocarpus var. geosporus (Nicolson & Gerdemann) Gerdemann & Trappe. I propose raising this variety to species rank, having discussed the matter with Dr. J. W. Gerdemann (University of Illinois, Champaign) and Dr. J. M. Trappe (U.S. Forest Service, Corvallis, Oregon), as well as with others working with the Endogonaceae. The differences between it and Gl. macrocarpus var. macrocarpus sensu Gerdemann & Trappe (1974) are at least as great as, for example, the differences between Gl. fasciculatus (Thaxter sensu Gerdemann) Gerdemann & Trappe and Gl. macrocarpus var. macrocarpus, or between Gl. microcarpus Tul. & Tul. and Gl. fasciculatus. Similarly, Gl. constrictus Trappe is as different from Gl. macrocarpus var. macrocarpus as is Gl. macrocarpus var. geosporus.

Varietal status implies a knowledge of the phylogenetic relationships within a genus and suggests a closer relationship between varieties than between species. In the present state of knowledge, we cannot yet make such interpretations. Suggesting that the two varieties of Gl. macrocarpus are more closely related than, for example, Gl. macrocarpus var. geosporus and Gl. constrictus, is not supported by the evidence available.

Nicolson & Gerdemann (1968) erected three varieties of Endogone macrocarpa (Tul. & Tul.) Tul. & Tul., based on resemblance to the specimens used by Thaxter (1922) in his classical contribution to

the taxonomy of the group. Later, Gerdemann & Trappe (1974) raised E. macrocarpa var. caledonia Nicolson & Gerdemann to full species status and placed it in the genus Glomus. The absence of sporocarps, the very dark spore walls, and the long extension of spore-wall thickening into the subtending hypha of Gl. macrocarpus var. geosporus are, I believe, sufficiently different from the features of Gl. macrocarpus var. macrocarpus to raise the former to the level of full species. The description following is based on an examination of the type collection in the Farlow Herbarium, Harvard, and of specimens collected from Iowa, Georgia, Arizona, and Mexico.

Glomus geosporus (Nicolson & Gerdemann) Walker stat. nov. (Figure 5.3)

= Endogone macrocarpa (Tul. & Tul.) Tul. & Tul. var.  
geospora Nicolson & Gerdemann. Mycologia 60:  
 318-319. 1968

= Glomus macrocarpus var. geosporus (Nicolson & Gerdemann)  
 Gerdemann & Trappe. Mycologia Memoir No. 5:55-56. 1974.

Sporocarps unknown. Chlamydo-spores formed singly in soil, globose to subglobose or broadly ellipsoid, 110-290  $\mu\text{m}$ , smooth and shiny or with a dull appearance, or roughened from adherent debris; light yellow-brown and transparent to translucent when young, becoming opaque and dark red-brown to black at maturity.

Spore wall 4-18  $\mu\text{m}$ , finely laminated, the outer lamination hyaline and transparent in very young spores. Wall often becoming perforated with age due to attack by microorganisms.

Figure 5.3. Chlamydospores of Glomus geosporus

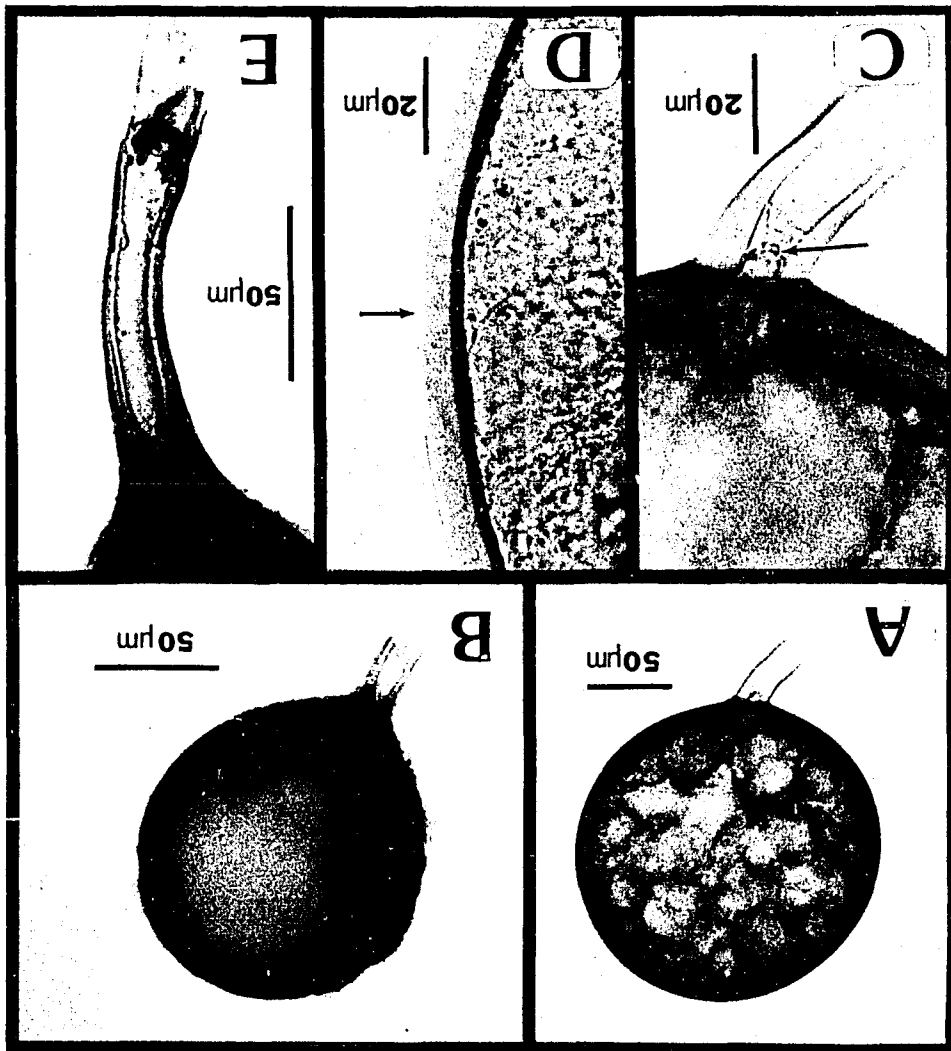
(A) A young spore (from the type collection) showing the spore contents.

(B) An opaque mature spore showing roughening from adherent debris.

(C) Detail of thickened subtending hypha with septum (arrowed). From the spore in Figure 5.3(B).

(D) Wall detail from a spore in the type collection. Note the hyaline outer lamination in this young specimen (arrowed).

(E) Greatly thickened laminated subtending hypha of a mature chlamydospore.



Spores with one straight, simple to slightly funnel-shaped hyphal attachment (or rarely two adjacent attachments) 10-24  $\mu\text{m}$  diam, the subtending hypha with yellow to dark yellow-brown wall thickening that extends 50-150  $\mu\text{m}$  along the hypha from the spore. Spore contents of uniform oil droplets cut off at first by a thick septum near the pore. This septum is later obscured by wall thickening at the base of the spore. Germination is by regrowth through the subtending hypha.

DISCUSSION OF GLOMUS GEOSPORUS

Glomus geosporus can be separated from similar species by the greatly thickened subtending hypha and translucent to opaque red-brown to black walls. Gl. macrocarpus is similar, though less deeply colored and with translucent to transparent walls, but has a much shorter subtending hypha with thickening extending only a short distance (usually 10-30  $\mu\text{m}$ ) from the spore base. Gl. epigaeus Daniels & Trappe has much brighter yellow to yellowish-brown spore walls and has even shorter thickening of the hyphal attachment, which is much smaller in diameter (10  $\mu\text{m}$  or less compared with 10-24  $\mu\text{m}$  for Gl. macrocarpus and 50-100  $\mu\text{m}$  for Gl. geosporus); it also has a characteristic insertion of the subtending hypha at the spore base (Daniels & Trappe, 1979). Gl. etunicatus is generally smaller (68-162  $\mu\text{m}$ ) than Gl. geosporus, and when young has a rough hyaline to subhyaline outer coat. Older spores of Gl. etunicatus that have this coat sloughed off may be confused for Gl. geosporus, but are lighter in color and have little wall thickening down the subtending hypha, which usually is less than 35  $\mu\text{m}$  long and often much less, breaking close to the base of the spore. Gl. constrictus resembles Gl. geosporus in wall characteristics, but has a distinctive constricted hyphal attachment which often is recurved and swollen and may be reminiscent of the suspensor cell of Gigaspora spp. (Trappe, 1977). Both species have red-brown to black, dull to shiny walls at maturity, though Gl. constrictus tends more to be

shiny and black. These two species are difficult to separate under a dissecting microscope. This problem was discussed by Trappe (1977).

Because the yellow-brown to red-brown, globose, subglobose, obovoid to ellipsoid spores of Glomus spp. are not easy to identify, the following key has been devised. Collections of hyaline to white spores, and brown spores which are mostly clearly ellipsoid will not key here, and the latter should be sent to a specialist for identification.

- 1        Spores mostly less than 55  $\mu\text{m}$  diameter.....Gl. microcarpus
- 1        Most spores greater than 50  $\mu\text{m}$ .....2
- 2(1)    Most spores 50-100  $\mu\text{m}$  diameter.....3
- 2        Most spores greater than 100  $\mu\text{m}$  diameter.....5
- 3(2)    Subtending hypha with wall thickening conspicuous and extending more than 30  $\mu\text{m}$  from base. Hyaline outer coat, if present, persistent.....4
- 3        Subtending hypha with little thickening (much less than 30  $\mu\text{m}$ ), often broken off close to the spore base and difficult to see. Young spores with an outer hyaline or subhyaline coat which sloughs off at maturity (examine several spores to observe the range of variation).....Gl. etunicatus
- 4(3)    Spores with a thin subhyaline outer coating (0.5-1  $\mu\text{m}$ ) not sloughing with age. Globose to subglobose or broadly ellipsoid. Some spores in a collection exceeding 110  $\mu\text{m}$  diam. Subtending hypha appearing inserted into the spore wall in at least some spores. Spores light yellow to red-brown.....Gl. epigaeus
- 4        Spores with or without an outer coating. Yellow to dark red-brown. Globose to subglobose, obovoid to irregular. Many spores much less than 100  $\mu\text{m}$  diam. Subtending hypha simple and straight (in some collections highly variable, swollen, recurved, funnel-shaped, etc.). Spores often in loose clusters, and may be in sporocarps.....Gl. fasciculatus GROUP

- 5(2) Spores mostly globose, yellow to light brown with an outer wall which is hyaline to subhyaline and sloughs with maturity (examine a series from a collection to see the range). Spores 68-144  $\mu\text{m}$  diam. Subtending hypha thickened for only a short distance (less than 30  $\mu\text{m}$ ) and either very thin-walled and hyaline or broken off close to the spore.....Gl. etunicatus
- 5 Spores with or without an outer hyaline to subhyaline coat. Hyaline outer coat, if present, persistent.....6
- 6(5) Spores red-brown to black (light yellow in some immature specimens) with the subtending hypha either conspicuously constricted at the base and somewhat bulbous, or with greatly thickened walls usually extending more than 30  $\mu\text{m}$  from the spore base.....7
- 6 Spores yellow-brown to light brown or light red-brown (in some collections a deep red-brown). Subtending hypha may be thickened, but not usually for more than about 30  $\mu\text{m}$ ...  
.....8
- 7(6) Spores with a conspicuous constriction in the somewhat swollen subtending hypha at the spore base, giving a bulbous appearance to the attachment. Hypha often broken off close to the spore, or recurved and difficult to see. Constriction often obscured by an accumulation of debris. Many spores shining black. Sometimes in loose clusters of 3-12 spores.....Gl. constrictus
- 7 Spores lacking a constricted subtending hypha. Subtending hypha with walls usually greatly thickened for 30-150  $\mu\text{m}$  from the spore base. Young spores closed by a thick septum which is later obscured by wall thickening. Most spores mat red-brown and opaque, though young spores may be light yellow-brown to red-brown and somewhat translucent.....Gl. geosporus
- 8(6) Spores up to 140 (occasionally up to 200)  $\mu\text{m}$  in diam. Globose to subglobose. Light yellow-brown to light brown with some collections deep red-brown. With a thin subhyaline outer coating (0.5-1  $\mu\text{m}$  thick). Some spores in a collection may be less than 90  $\mu\text{m}$ . Subtending hypha little thickened and appearing inserted into the spore wall in at least some specimens.....Gl. epigaeus



- 8 Spores almost all greater than 100  $\mu\text{m}$ , reaching 230  $\mu\text{m}$  (usual range 90-200  $\mu\text{m}$ ). Globose to subglobose, occasionally ellipsoid or obovoid. Subtending hypha with wall thickening obvious, but rarely extending more than 10-30  $\mu\text{m}$  from the spore base, even though the hypha usually is much longer than this. Lacking any hyaline or subhyaline outer coat, though sometimes roughened from adhering debris. Often found in loose clusters or sporocarps, as well as singly.....Gl. macrocarpus

## ACKNOWLEDGMENTS

I wish to thank Dr. James M. Trappe for his helpful suggestions and discussions, and for allowing me to examine his Mexican collection; for preparing the Latin diagnosis; and for suggesting the specific epithet for Glomus occultus. Dr. H. E. Bloss, University of Arizona, sent specimens of Gl. geosporus from Yuma, Arizona, and I thank him for them. Thanks also to the Keeper of the Farlow Herbarium for loaning the type of Gl. geosporus.

Joan Zito and Kathleen Bason, Iowa State University, prepared the photographic plates and deserve thanks for their painstaking efforts.

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## PART 6.

GLOMUS ALBIDUS:

A NEW SPECIES IN THE ENDOGONACEAE

Glomus albidus: a new species in the Endogonaceae

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Prepared for submission to Mycotaxon.

## INTRODUCTION

Separate investigations into the Endogonaceae and endomycorrhizae of Iowa and Ohio yielded the same undescribed species. This species has many of the characteristics ascribed to the "white reticulate" spore types of El-Giahmi et al. (1976), Hayman (1978), Mosse (1972), and Mosse & Bowen (1968a, 1968b). The spores are white, have a poorly defined subtending hypha at maturity, appear somewhat "reticulate" when viewed through a compound microscope, and germinate by production of a germ tube through the spore wall. The species is herein named Glomus albidus sp. nov. The Ohio isolate was established in pot culture on corn, and specimens from two such cultures were used for the type collection. The paratype is from a field collection from central Iowa.

GLOMUS ALBIDUS Walker & Rhodes sp. nov.

(Figure 6.1)

[LATIN DIAGNOSIS]<sup>1</sup>

Sporocarps unknown. Chlamydospores under reflected light hyaline when young, white to off-white at maturity, always appearing yellowish to brownish-yellow by transmitted light through a compound light microscope. Spores with one subtending hypha (rarely with two subtending hyphae), borne singly in the soil on coenocytic hyphae. Mature spores (85-)95-168(-198) x (85-)95-168(-177)  $\mu\text{m}$ , globose to subglobose, occasionally ovoid or irregular: cyanophilous in cotton blue at maturity, slowly and less strongly so in youth; mature spores becoming dull orange to yellow in Melzer's reagent, young spores becoming pink to orange-red.

Spore walls continuous with hyphal walls, clearly double in youth, consisting of an outer hyaline wall 0.5-2  $\mu\text{m}$  thick, and an inner subequal finely laminated wall, light yellow and 0.5-2  $\mu\text{m}$  thick. At maturity, the outer wall crumbling and expanding, becoming as much as 8  $\mu\text{m}$  thick in places and rendering the spore opaque; then partly sloughing off, often becoming less than 1  $\mu\text{m}$  thick and having a roughened granular appearance.

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<sup>1</sup>Latin diagnosis omitted so that this does not constitute a legal description under the rules of the International Nomenclature Committee.

Figure 6.1. Chlamydospores of Glomus albidus

(A) Mature spore (left) and immature spore (right) in juxtaposition.

(B) Young spore of Gl. albidus. Note the two distinct walls.

(C) A mature spore with the outer wall expanded (top) and sloughed (base).

(D) Germinating spore. The germ tube (gt) has grown directly through the spore wall. The collapsed subtending hypha (h) is on the left.

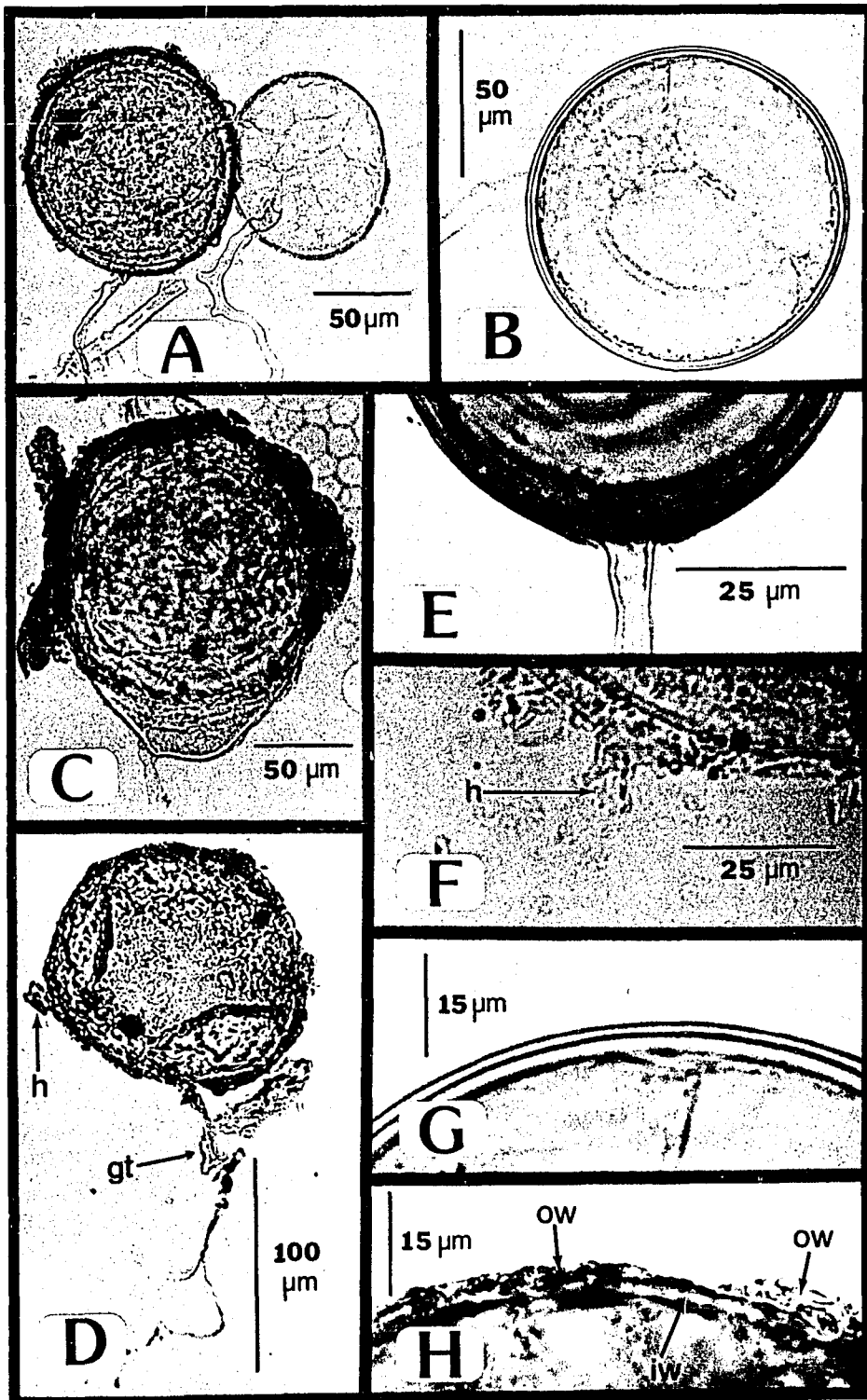
(E) Detail of subtending hypha of a young spore. Note the two walls.

(F) Detail of subtending hypha of an old spore. The outer wall has disappeared and the hypha (h) has almost collapsed.

(G) Wall structure of a young spore. Two layers can clearly be seen.

(H) Wall structure of an old spore. The inner wall (iw) is intact, but the outer wall (ow) has crumbled and is breaking down.





Subtending hyphae 2-walled, outer wall thickened at spore base, (3-)5-15  $\mu\text{m}$  wide, usually straight and simple, but sometimes constricted at the spore base or expanded by thickening of the outer wall to become slightly funnel-shaped. Occasionally with a bulging septum 5-20  $\mu\text{m}$  distad of the pore, but usually open. Outer wall up to 0.7  $\mu\text{m}$  thick, sloughing at maturity to leave the inner wall (0.2-0.5  $\mu\text{m}$  thick) unsupported; hypha then shrivelling and collapsing, often becoming difficult to see.

Spore contents of crowded oil droplets, usually becoming angular from mutual pressure to give a reticulate appearance: seemingly sealed off by collapse of the subtending hypha at maturity.

Germination by germ tube penetrating the spore wall. Regrowth of the subtending hypha not observed.

#### Distribution and habitat

Known from the rhizosphere of winter wheat (Triticum aestivum L.) in Ohio, and from around the roots of grasses (Setaria spp. and Bromus inermis Leyss.) and poplars (Populus spp.) in an old meadow site in central Iowa. Found throughout the growing season. Probably also present in winter as resting spores in the soil and mycelium in living roots.

#### Mycorrhizal associations

Forming vesicular-arbuscular mycorrhizae with corn (Zea mays L.),

onion (Allium cepa L.), sorghum (Sorghum vulgare Pers.), and poplar (Populus x euroamericana (Dode) Guinier). Associated in the field with mycorrhizal roots of poplars, foxtail grasses (Setaria spp.), smooth brome (B. inermis), and wheat (T. aestivum).

#### Etymology

Latin, albidus; whitish. Referring to the white to off-white appearance of the spores when viewed by reflected light.

#### Collections examined

HOLOTYPE: OHIO -- Pickaway Co. (Collected by L. H. Rhodes, 6.vi.1977) among roots of winter wheat (T. aestivum). Type specimens from two pot cultures on corn (Zea mays), Walker #169, 170 (OSC; isotype FH, ISC). PARATYPE: IOWA -- Marshall Co., Rhodes, at the Iowa State University Rhodes Farm, Walker #179, 23.viii.1978 (ISC).

In addition, specimens from the Rhodes Farm were examined from random soil samples taken every two weeks during the summer and early autumn of 1978 as part of a population dynamics study of endogonaceous spores.

## DISCUSSION

Mature spores of Glomus albidus are separated from those of other Glomus species by their white to off-white color and by their thin-walled, collapsed subtending hyphae. The change of color to yellowish when viewed through a compound microscope is also characteristic. Gl. gerdemannii Rose, Daniels & Trappe also has a very delicate subtending hypha, but, unlike Gl. albidus, the hypha is thickened for a short distance from the point of attachment. The outer wall of Gl. albidus and the middle wall of Gl. gerdemannii both appear firm in youth and then swell and break down as the spore matures. However, the breakdown of the former is into granular material, whereas the latter is into flaky pieces of laminations. Glomus clarus Nicolson & Schenck, and Gl. occultus Walker sp. ined. have hyaline to white spores possessing an outer coat which sloughs off with maturity. Both these species, however, lack the consistently whitish color of Gl. albidus at maturity and have well-defined subtending hyphae at all stages of development (Nicolson & Schenck, 1979; C. Walker, Iowa State University, in prep.).

Young spores of Gl. albidus are hyaline and can be confused with those of Gl. occultus, Gl. clarus, and Gl. pallidus Hall. Glomus occultus has a more complex wall structure than Gl. albidus, consisting of three layers, is generally much smaller (35-100 x 40-120  $\mu\text{m}$ ), and has a persistent subtending hypha which lacks an outer wall. The subtending hypha of Gl. occultus often is recurved,

whereas the subtending hyphae of Gl. albidus usually is straight. The walls of Gl. clarus spores are not of equal thickness, the outer wall being much thicker than the inner. The two walls of young spores of Gl. albidus are of almost equal thickness. In addition, young spores of Gl. clarus have a thin outer coat, tightly adhering to the outer wall, thus making three layers in all. The description of Gl. pallidus indicates that only one, laminated wall is present in that species (Hall, 1977), and even mature spores are much smaller than many of the immature specimens of Gl. albidus to be found in a collection. Finally, young spores of Gl. albidus have a characteristic pink to orange-red reaction to Melzer's reagent. Gl. clarus, Gl. occultus, and Gl. gerdemannii have no such reaction. The response of Gl. pallidus to this reagent is unknown.

Careful observation of a series of spores will allow all stages of development to be studied, making identification easier and more certain.

Glomus albidus is probably one of the "white reticulate" isolates referred to in the literature. However, Hall & Fish (1979) refer to Hayman's (1978) "white reticulate" species, and state that it is not the same as that of Mosse & Bowen (1968a, 1968b). The key of Hall & Fish (1979) indicates that Hayman's spore has projections 12-30  $\mu\text{m}$  high on the outer wall. No such structures occur on Gl. albidus, and therefore it is not the same as that observed by Hayman (1978). The "white reticulate" spores described by Mosse &

Bowen (1968a) apparently are similar to Gl. albidus, but one of us (Walker) has received spores from G. D. Bowen of an Australian "white reticulate" isolate, and these are not Gl. albidus. The "white reticulate" spores of El-Giahmi et al. (1976) and Mosse (1972) look very similar to Gl. albidus, but the descriptions are insufficient for conclusive identification. It is possible that several taxa have spores that could fall into the general morphological category of "white reticulate."

Consideration was given to raising a new genus to accommodate chlamydosporic species of the Endogonaceae that germinate through the spore wall, but there are good reasons for not doing so. The germination of many Glomus spp. has not been observed, and it therefore would be impossible to place such species to genus if germination mode was used as the sole generic criterion. At least one other species in Glomus (Gl. pallidus) is known to germinate through the spore wall (Hall, 1977), but in all other respects it clearly is a Glomus sp. In the genus Gigaspora, there are two germination modes viz., with or without compartmentalization of spore contents prior to germ tube egress, yet there seems no justification for splitting the genus on germination characteristics. If Glomus were separated on such features, the same might justifiably be done for Gigaspora. It may be that, as more is learned about members of the Endogonaceae, subgenera will be erected based on mode of germination. The use of such characters

for generic delimitation at present would lead to confusion rather than clarification.

Rose et al. (1979) referred to the similarities between Gl. gerdemannii and azygosporic species in Gigaspora and Acaulospora. There are two characteristics of Gl. albidus that could similarly be considered as evidence for a link between it and azygosporic species in the family. The germination directly through the spore wall, rather than by regrowth through the subtending hypha, is similar to that of Gigaspora and Acaulospora; and the reaction to Melzer's reagent is typical of the white-spored species in these two genera. However, there is no evidence of sexual or pseudo-sexual structures, such as the thin-walled hyphae on the mother vesicle of Acaulospora spp., or the small hyphal projection on the bulbous suspensor-like cell of Gigaspora.

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## OVERALL SUMMARY AND DISCUSSION

General

This dissertation presents a general introduction to the main types of mycorrhizae, a synopsis of the Endogonaceae in central Iowa with a presentation of taxonomic concepts used in that family, and a review of literature relating to the mycorrhizae of poplars. Also presented are papers describing new species in the Endogonaceae, and two reports of studies on endogonaceous spores associated with poplar trees in Iowa.

Poplar mycorrhizae

Part 1 of the dissertation reveals that, in seven clones of hybrid poplars potted in five different Iowan soils, all three main types of mycorrhizae developed. Vesicular endomycorrhizae were most common, but ecto- and ectendomycorrhizae occurred also on two of the clones. A possible relationship between the sections of the genus Populus and mycorrhizal status was noted, although more studies would be required to ascertain this relationship. Five of the clones used in the work for Part 1 were P. x euroamericana. None of these clones formed ectomycorrhizae in this instance. The possibility of an inverse relationship between endomycorrhizal formation and ectomycorrhizal formation was raised. Thus, the answer to the first part of question 2 on page 4 ("What types of

mycorrhizae are found on these poplars ...?") is provided.

The first question raised on page 4 ("Are poplars used for intensive culture mycorrhizal?") is answered by a qualified "yes." Although the work of Part 1 showed that mycorrhizae could form in the poplars examined, outplanting experiments resulted in minimal mycorrhizal formation (see Part 2 herein). Why this was so is unknown. Further work on establishing mycorrhizae in poplars and assessing resultant growth effects would be worthwhile.

If mycorrhizae are beneficial to the trees, and if establishment of the symbiosis in the field is consistently poor, then research on instigation of mycorrhizal colonization prior to outplanting would be worthwhile.

#### Population diversity of endogonaceous spores

The diversity of species in the Endogonaceae in Iowan soils was high. Previous reports have indicated that 1-4 species commonly occur in any one site (Table 7). In Part 1 of this work, the five soils from Iowa showed between 5 and 7 species per soil (Table 1.4), although because of the nature of the survey, it is not possible to be certain that species not recorded did not occur in the soils. In Part 2, data from two field sites are presented. A site at the 4H Camping Center, Boone Co., Iowa had 10 endogonaceous species, and a location near Rhodes, Iowa had 12 (Table 2.1). The previous largest number of species recorded from one site was 6 (Hayman, 1978;

Thapar & Khan, 1973). This apparent disparity with other reports may be partly an artifact of spore extraction methods. Many workers have used sieves with openings no smaller than 100  $\mu\text{m}$  during spore extraction. Several of the species of Endogonaceae found in Iowa have spores less than 100  $\mu\text{m}$  maximum diameter, and if such is the case in other locations, the number of species reported would have been lower than are actually present.

#### Effects of site preparation on endogonaceous spores

Evidence from the Rhodes site (Part 2 herein) indicated that, for most endogonaceous species at that location, there was a slight but non-significant depression in spore production. Only for one species, Glomus albidus, was a significant effect noted. With that species, spore populations were depressed after application of herbicides. However, the species was not eliminated, and, providing the spores remained viable, it is unlikely that establishment of mycorrhizae would be prevented by this depression of spore numbers. Further research on the effects of herbicides, both on spore populations and spore viability, would be worthwhile.

#### Distribution and dynamics of endogonaceous spore populations

Results of an examination of the dynamics of endogonaceous spore populations in the two sites (4H Camping Center and Iowa State University Rhodes Farm) in central Iowa were reported (Part 2 herein),

as was the distribution of spores within sites. The changes in population levels closely followed changes in soil moisture.

Correlation coefficients were calculated and a positive relationship (significant at  $p \leq 0.05$ ) was established. But, this relationship apparently existed only for a certain range of moisture levels. If the values for spore numbers and moisture measured during the drought were included, the correlation no longer existed. The suggestion was therefore made that a threshold moisture value existed, below which sporulation and germination did not take place. Further research would be worthwhile on this aspect of mycorrhizal biology.

Examination of overall spore populations indicated a trend of lower spore numbers in early summer, followed by a peak in autumn. This agrees with findings reported previously (Khan, 1974; Mason, 1964; Saif, 1977; Sutton & Barron, 1972), and possibly is related to root senescence.

The distribution of spores in the soil at each sampling time and over all sampling times was investigated, and shown to be variable. The variation over time was least; the same species being found in the same plots at most sampling times (Figures 2.1 and 2.2). However, some species (e.g., Glomus constrictus at the 4H site; and Gigaspora gilmorei and Acaulospora spinosa at Rhodes) appeared intermittently. Variation over space was large for both species and population levels (Figures 2.1, 2.2, and 2.3). The need for extensive sampling in order to obtain reliable information on spore populations

clearly is indicated by this work.

The third question on page 4 asked how populations of endogonaceous spores changed during the initial establishment of intensive culture populations of poplar. The information given in Part 2 herein may not be a relevant answer to that question. Because the poplars apparently failed to become mycorrhizal, the changes in populations of spores cannot be related to the poplars. The data do show, however, that spores of the Endogonaceae are present in large numbers in the soil throughout the growing season, even after disruption of the ground cover by plowing and herbicide treatment.

#### Species of Endogonaceae in Iowa

One new species in the genus Acaulospora (Part 4 herein) and three in the genus Glomus (Parts 5 and 6 herein) are described as part of the work for this dissertation. One taxon in Glomus is given new status (Glomus macrocarpus var geosporus is raised to species level) (Part 5 herein), and a new, monospecific genus (Complexipes) is erected (Part 3 herein).

Acaulospora spinosa Walker & Trappe sp. ined. is known from two sites in central Iowa, and from Mexico. Interestingly, the spores of this species are abundant at one of the Iowa sites (4H), but uncommon at the other (Rhodes). Why this should be so is unknown. Vesicular-arbuscular mycorrhizae have been formed in strawberry with this species. Attempts to establish similar symbioses with poplars are in progress.

Glomus occultus Walker sp. ined. sporulates in great abundance in soil at the Rhodes site, and forms endomycorrhizae in pot culture with Populus x euramericana. Glomus albidus Walker & Rhodes sp. ined. is a white-spored species, also found in Ohio, which forms endomycorrhizae on P. x euramericana and also on other plants.

Complexipes moniliformis Walker gen. et sp. ined., the type species for a new genus, is not known to form endomycorrhizae, but forms ectendomycorrhizae with pines (Wilcox et al., 1974). The type collection of this fungus is from a small pine plantation in Ames, Iowa.

The list of endogonaceous species identified from Iowa during this work includes 22 species in 6 genera (Table 8). In addition, I have found fragments of sporocarps which possibly are Endogone lactiflua and Modicella malleola. The differences among the soils examined indicate that further sampling in other locations may add more species to the list.

The second part of question 2 (page 4) is answered only in part. ("... is it possible to identify the fungal symbionts?")

Cenococcum geophilum almost certainly is ectomycorrhizal with some of the poplars (Part 1 herein). Glomus occultus and Gl. albidus have been established in pot culture with one of the clones of P. x euramericana (Canada Blanc: see Table 1.1 for details of clones). The same clone planted in pots of soil from the 4H site and the Rhodes site became endomycorrhizal and mixed endogonaceous spore populations occurred in

Table 8. Species of Endogonaceae found in central Iowa

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ACAULOSPORA spp.A. scrobiculataA. spinosa sp. ined.A. trappeiCOMPLEXIPES sp.C. moniliformis gen. et sp. ined.ENTROPHOSPORA sp.E. infrequensGIGASPORA spp.Gi. calosporaGi. giganteaGi. gilmoreiGi. heterogamaGi. roseaGLOMUS spp.Gl. albidus sp. ined.Gl. constrictusGl. epigaeusGl. etunicatusGl. geosporus stat. ined.Gl. fasciculatusGl. macrocarpusGl. microcarpusGl. mosseaeGl. occultus sp. ined.SCLEROCYSTIS spp.S. rubiformisS. sinuosa

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the pots. However, it will be necessary to isolate species in pot cultures to determine which fungi are symbiotic with the trees. In view of the wide host range of most species of Endogonaceae (Gerdemann & Trappe, 1974), it is likely that all the species known from Iowa, with the exception of Complexipes moniliiformis and Entrophospora infrequens, will be able to form phycomycetous endomycorrhizae with poplars. Future work might be directed to establishing pot cultures from single spores of the fungi found at the 4H site and at the Rhodes site, and at using the derived cultures to establish mycorrhizae of poplars. Assessment of growth effects of the different symbioses could then be made.



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